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EFFECT OF ALFALFA ON THE FERTILITY ELEMENTS OF THE SOIL IN COMPARISON WITH GRAIN CROPS[1]

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INTRODUCTION

That continuous growing of grain crops decreases the fertility of the soil, and that the growing of legumes in a measure restores this fertility are facts well known to students of soil science. To what degree this takes place is not so well known.

Alfalfa is one of the important crops of Kansas, and was one of the first to be grown. Most of the work concerning the effect of legumes on soil nitrogen has been with red clover and legumes other than alfalfa. This is partly due to the fact that in those states and the countries where investigations have been conducted, alfalfa is not a common crop.

The main problem of the present investigation is this: When alfalfa has been grown continuously on a piece of land, and all the crop harvested as hay, how much nitrogen and organic matter has been added to the soil? Phosphorus and calcium were also included in the study.

There are in Kansas a large number of fields where alfalfa has been grown from 20 to 30 years and near by are others which are still in native sod and also some which have been cropped continuously to grain since the native sod was broken. It was thought that a study of the fertility of the cropped fields as compared with those still in native sod would give valuable information. It will be well to consider first the present status of knowledge on this subject.

HISTORICAL

That native legumes are important sources of nitrogen in the native soil has been shown by several investigators. Warren (10) in a study of the native vegetation of Kansas and Nebraska found that on the average there were 17 native legumes to the square yard. Most of them have enormous root systems. Warren attempted to ascertain the prevalence of nodules on different species and found large numbers on every one. Also he found that the native legumes were more abundant on the poorer soils. This appears

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to show that after the legumes have enriched the soil, other plants crowd them out. In pastures the legumes tend to disappear because they are so readily eaten by live stock.

Alway and Pinckney (2) found in four species of native legumes, an average nitrogen content of 2 per cent. They estimated that the natural growth of legumes could furnish 10 pounds of nitrogen per acre annually.

According to Russel, a plat on Broadbalk field, Rothamstead, which was not cropped after 1882, gained 91.7 pounds of nitrogen per acre per annum. This plot contained 3.32 per cent $CaCO_3$. Another plat which contained only 0.16 per cent $CaCO_3$, gained 60 pounds of nitrogen per acre per annum. These amounts are figured to the depth of 27 inches (5). On Doctor Gilbert's meadow which was laid down to grass in 1856, and mowed annually, the amount of nitrogen was doubled in the top 9 inches in a little over 50 years (5).

Russel (4) mentions the classic instance of the restoration of the nitrogen content of the soil of the Schultz-Lupitz estate in Germany as an example, of the power of legumes to restore nitrogen to worn-out soil. This estate was once a barren sand. Lime and fertilizers containing phosphorus and potassium were used but no nitrogenous manures. The land was cropped alternately to lupines and cereals, the former being either plowed under or fed. The barren sand after a time became a rich soil capable of producing a large variety of crops. As shown by Russel the enrichment in nitrogen of soils by legumes is dependent upon available phosphorus and potassium. Thus in experiments at the Rothamstead station there was a large increase in the nitrogen content of soils in which clover was grown in rotation with other crops and to which potash and superphosphates but no nitrogenous manures had been added. There was a loss in nitrogen with the same cropping when no potash or superphosphates were applied. As the experiments were conducted over twenty years the results are no doubt significant.

Shutt (6) found that after 22 years of cultivation a prairie soil of Western Canada had lost a total of 2190 pounds of nitrogen per acre in the top 8 inches. Of this only 700 pounds were contained in the crops removed from the soil. Snyder (7) determined the loss of nitrogen in the soil of a number of typical Minnesota farms in a 10-year period. "The loss from grain farms was from 3 to 5 times the amount removed by the crops. This loss was attributed to the rapid decay of humus and the liberation of nitrogen." The nitrogen content in all these soils was high. Whitson, Stoddart and McLeod (12) collected a number of samples of both cropped and virgin soil from Wisconsin farms. The average nitrogen content of the cropped soil was 0.107 per cent and of the virgin soil 0.170 per cent, showing a loss of over one-third the original stock of nitrogen. These authors also estimated the amount of nitrogen removed by crops and that added by manure and clover. The nitrogen not accounted for by crop removal after correcting for that added in the manure and the clover, was assumed to be lost by leaching and denitrification.

In 6 of the 21 cases, the difference in the amount of nitrogen in the virgin soil and in the cropped soil was not enough to account for the nitrogen which had been removed in the crop. It was suggested that this difference was accounted for by the fixation of nitrogen by soil bacteria. The average amount lost by leaching and denitrification amounted to only 22.3 per cent of that removed by the crops. In clay soils more than four-fifths of the total loss of nitrogen was removed by crops.

In continuing these studies, the authors (13) found that the largest losses of nitrogen occurred in those soils which have a rather large per cent of total nitrogen. In 16 of the 21 cases in which the virgin soil contained 0.2 per cent of nitrogen or over, the loss above that removed by crop exceeded 500 pounds per acre. In 21 of 26 cases where the per cent of nitrogen in the virgin soil was less than 0.2 per cent, the loss was less than 500 pounds per acre. The loss from manured soil was greater than where soils were not manured.

Alway (1) concluded that in the soils of Nebraska, the only constituents that have declined appreciably under cultivation are nitrogen and total organic matter. Whitson and Stoddart (11) found a decrease in the phosphorus content in virgin soil. They found that the average content of phosphoric acid, expressed as P_2O_5 was 0.185 per cent and in nine adjoining cropped soils it was 0.120 per cent, indicating a loss of one-third of the original stock of phosphorus.

It appears that cultivating the soil does not always cause a decrease of nitrogen. Stewart (8) analyzed a number of Utah soils and found an average of 2055 pounds of nitrogen per acre to a depth of 12 inches in soil that had been cropped to wheat, as compared with 2009 pounds in fields that had been cropped to alfalfa and 1984 pounds in virgin soil.

Stewart explains these results by a difference in the composition of the virgin soil, an increase in nitrogen in the cultivated soils due to azotobacter and to a rise of nitrogen from the subsoil by deep roots.

That crop production may be increased after the growth of alfalfa even if the stock of nitrogen has not been materially increased, is shown by an experiment reported by Lyon and Bizzell (3).

Alfalfa and timothy were grown for 6 years on adjoining plats. On plowing up they were planted to corn the first year and oats the second. The yield of corn on the alfalfa plat was 63 bushels per acre, and on the timothy plat 47 bushels per acre. The oats yielded 26 bushels on the alfalfa plat and 27 on the timothy plat. Analyses of the soils from the two plats showed that the alfalfa plat contained not to exceed 0.01 per cent more nitrogen than the soil from the timothy plat. This would amount to 250 pounds per acre if the soil to the depth of 8 inches is assumed to weigh 2,500,000 pounds.

Very little work has been done relating to the effect of legumes on the mineral elements of the soil. Certain work has shown that some elements, particularly phosphorus, have decreased. Other work fails to show any appreciable change. This is partly due to the inherent difficulties of the

TABLE I

Analysis of soils in virgin sod, fields cropped to alfalfa and of fields cropped to grain in Kansas

Location, county and land owner	Cropping, rotation, character and type of soil	Depth of section	Lab. No. numbers	Nitrogen	Phos. phorus	Calcium	Organic matter	Inorganic matter
		inches		per cent	per cent	per cent	per cent	per cent
Leavenworth County		Humid section						
N. W. 10 of N. W. 40 of N. W., Sec. 24, T. 10 S., R. 22 E., of 6 p. m.	Alfalfa 8 years, Blue Grass 10 years before alfalfa, Brown silt loam.	Soil 0 7 Subsurface 7 20 Subsoil 20 40 Subsoil 40 80	1310.1 1310.2 1310.3 1310.4	0.163 0.094 0.061 0.040	0.043 0.040 0.040 0.038	0.860 0.480 0.040 0.010	1.610 0.940 0.360 0.190	0.080
N. W. 10 of S. W. 40 of S. W., Sec. 24, Twp. 10 S., R. 22 E., of 6 p. m.	Cropped to wheat. Well farmed Brown silt loam.	Soil 0 7 Subsurface 7 20 Subsoil 20 40 Subsoil 40 80	1317.1 1317.2 1317.3 1317.4	0.174 0.130 0.044 0.052	0.048 0.041 0.034 0.044	0.860 0.360 0.010 0.060	1.870 1.260 0.100 0.070	0.070
N. W. 10 of N. E. 40 of N. E., Sec. 18, Twp. 9 S., R. 21 E., of 6 p. m.	Alfalfa 14 years, before that corn and wheat, Brown silt loam.	Soil 0 7 Subsurface 7 20 Subsoil 20 40 Subsoil 40 80 Subsoil 80 120	1318.1 1318.2 1318.3 1318.4 1318.5	0.222 0.177 0.121 lost 0.080	0.052 0.043 0.074 0.042	0.860 0.480 0.860 0.410	2.860 2.180 1.040 0.120	0.040
S. E. 10 of N. E. 40 of N. E. Sec. 5. Twp. 9 S. R. 21	Native meadow, Brown silt loam	Soil 0 7 Subsurface 7 20 Subsoil 20 40	1319.1 1319.2 1319.3	0.206 0.225 0.144	0.061 0.057 0.056	0.860 0.860 0.860	3.760 2.840 1.120	

Brown County

Location	Description	Depth	No.					
N. E. 10 of N. W. 40 of S. E. ¼ sec. 34. Twp. 1 N. R. 15 E. of 6 p.m.	Alfalfa 28 years. Marshall silt loam.	Soil 0–7	1768.1	0.211	0.063	0.770	2.280	0.770
		Subsurface 7–20	1768.2	0.142	0.055	0.680	1.540	0.680
		Subsoil 20–40	1768.3	0.062	0.059	0.760	0.400	0.760
		Subsoil 40–80	1768.4	0.031	0.066	0.810	0.060	0.810
S. E. 10 of S. E. 40 of S. E. ¼ sec. 28. Twp. 1 N. R. 15 E. of 6 p.m.	White clover and blue grass. Marshall silt loam.	Soil 0–7	1770.1	0.228	0.061	0.620	2.860	
		Subsurface 7–20	1770.2	0.133	0.051	0.610	1.450	
		Subsoil 20–40	1770.3	0.073	0.051	0.710	0.680	
		Subsoil 40–80	1770.4	0.047	0.049	0.710	0.310	
S. W. 10 of S. E. 40 of N. W. ¼ sec. 34. Twp. 1. N. R. 15. E. of 6 p.m.	Continuous grain cropping. Marshall silt loam.	Soil 0–7	1769.1	0.160	0.048	0.610	1.940	
		Subsurface 7–20	1769.2	0.135	0.048	0.660	1.510	
		Subsoil 20–40	1769.3	0.066	0.053	0.710	0.610	
		Subsoil 40–80	1769.4	0.031	0.066	0.860	0.096	

Nemaha County

Location	Description	Depth	No.					
N. W. 10 of S. E. 40 of S. E. ¼ sec. 8. Twp. 5 S. R. 14. E. of 6 p.m.	Alfalfa 21 years. Brown silt loam.	Soil 0–7	1765.1	0.170	0.039	0.660	1.690	
		Subsurface 7–20	1765.2	0.080	0.031	0.700	0.900	
		Subsoil 20–40	1765.3	0.032	0.049	2.000	0.230	0.280
		Subsoil 40–80	1765.4	0.008	0.061	5.280	1.490	
N. W. 10 of S. E. 40 of S. E. ¼ sec. 8. Twp. 5 S. R. 14. E. of 6 p.m.	Cultivated about 45 yrs. Brown silt loam to be compared with 1765.	Soil 0–7	1766.1	0.102	0.040	0.860	1.130	0.030
		Subsurface 7–20	1766.2	0.055	0.044	2.890	0.980	0.620
		Subsoil 20–40	1766.3	0.024	0.050	7.400	1.350	0.960
		Subsoil 40–80	1766.4	0.005	0.059	6.400	0.050	1.650
S. E. 10 of N. E. 40 of N. E. ¼ sec. 17. Twp. 5 E. R. 14. E. of 6 p.m.	Virgin pasture land. Some white clover and blue grass. Brown silt loam.	Soil 0–7	1767.1	0.181	0.038	0.840	1.970	0.050
		Subsurface 7–20	1767.2	0.085	0.042	1.860	0.900	0.240
		Subsoil 20–40	1767.3	0.028	0.049	7.000	0.460	1.940
		Subsoil 40–80	1767.4	0.008	0.058	6.810	0.280	1.680

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen per cent	Phosphorus per cent	Calcium per cent	Organic carbon per cent	Inorganic carbon per cent
Montgomery County								
S. E. 10 of S. W. 40 of N. W. ¼ sec. 1. Twp. 33 S. R. 14 E. of 6 p.m.	Alfalfa 13 years. Oswego silt loam.	Soil 0–7	1294.1	0.131	0.036	0.429	2.070	
		Subsurface 7–20	1294.2	0.106	0.022	0.485	1.350	
		Subsoil a 20–40	1294.3	0.054	0.029	0.506	0.800	
		Subsoil b 40–80	1294.4	0.031	0.032	0.712	0.520	0.020
		Subsoil c 80–120	1294.5	0.045	0.051	0.774	0.400	·0.030
N. E. 10 of N. W. 40 of S. E. ¼ sec. 1. Twp. 33 S. R. 1. E. of 6 p.m.	Native meadow. Oswego silt loam. To be compared with 1294. This land lies a little lower than 1294 and 1295.	Soil 0–7	1296.1	0.186	0.046	0.580	2.590	
		Subsurface 7–20	1296.2	0.114	0.032	0.630	1.640	
		Subsoil a 20–40	1296.3	0.067	0.037	0.530	0.910	
		Subsoil b 40–72	1296.4	0.024	0.056	0.130	0.350	0.160
S. E. 10 of S. W. 40 of N. W. ¼ sec. 1. Twp. 33 S. R. 14. E. of 6 p.m.	Kafir, corn, and grain for about 35 years. Oswego silt loam. To be compared with 1294.	Soil 0–7	1295.1	0.110	0.034	0.510	1.730	
		Subsurface 7–20	1295.2	0.094	0.028	0.570	1.250	
		Subsoil a 20–40	1295.3	0.050	0.025	0.640	0.710	0.010
		Subsoil b 40–80	1295.4	0.039	0.022	1.010	0.320	0.110
		Subsoil c 80–120	1295.5	0.049	0.055	0.750	0.260	0.030
S. E. 10 of N. E. 40 of S. E. ¼ sec. 13. Twp. 33 S. R. 16. E. of 6 p.m.	Alfalfa 10 years. Oswego silt loam.	Soil 0–7	1297.1	0.168	0.043	1.410	2.320	0.160
		Subsurface 7–20	1297.2	0.087	0.034	1.190	1.150	0.070
		Subsoil a 20–40	1297.3	0.038	0.023	2.170	0.700	0.430
		Subsoil b 40–72	1297.4	0.028	0.027	1.740	0.460	0.310
S. E. 10 of N. E. 40 of S. E. ¼ sec. 13. Twp. 33 S. R. 16. E. of 6 p.m.	General grain and corn farming. Oswego silt loam. To be compared with 1297.	Soil 0–7	1298.1	0.135	0.038	1.050	2.050	0.060
		Subsurface 7–20	1298.2	0.076	0.016	1.930	1.130	0.290
		Subsoil a 20–40	1298.3	0.043	0.026	2.460	0.700	0.500
		Subsoil b 40–72	1298.4	0.026	0.028	1.330	0.410	0.170

0.040

Location	Description	Horizon	Depth	No.				
S. E. 10 of S. E. 40 of S. E. ¼ sec. 10. Twp. 25 S. R. 5. E. of 6 p.m.	Corn for over 32 years. Osage silt loam. To be compared with 1897.	Soil	0–7	1896.1	0.139	0.027	0.490	1.540
		Subsurface	7–20	1896.2	0.117	0.031	0.480	1.240
		Subsoil a	20–40	1896.3	0.087	0.031	0.500	0.920
		Subsoil b	40–80	1896.4	0.057	0.032	0.500	0.580
S. E. 10 of N. E. 40 of S. E. ¼ sec. 10. Twp. 25 S. R. 5. E. of 6 p.m.	Native pasture. Osage silt loam. To be compared with 1897.	Soil	0–7	1898.1	0.196	0.035	0.580	2.350
		Subsurface	7–20	1898.2	0.132	0.035	0.530	1.400
		Subsoil a	20–40	1898.3	0.077	0.029	0.540	0.890
		Subsoil b	40–80	1898.4	0.067	0.026	0.550	0.690
N. E. 10 of S. E. 40 of S. E. ¼ S. E. sec. 10. Twp. 25. R. 5. E. of 6 p.m.	Corn over 20 years, then Alfalfa about 12 years. Osage silt loam.	Soil	0–7	1897.1	0.161	0.038	0.500	1.710
		Subsurface	7–20	1897.2	0.118	0.040	0.460	1.130
		Subsoil a	20–40	1897.3	0.071	0.028	0.460	1.070
		Subsoil b	40–50	1897.4	0.070	0.030	0.580	0.630

Chase County

Location	Description	Horizon	Depth	No.				
N. W. 10 of N. E. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Alfalfa about 25 years. Osage loamy silt loam.	Soil	0–7	1900.1	0.201	0.039	0.840	2.230
		Subsurface	7–20	1900.2	0.129	0.041	0.660	1.530
		Subsoil a	20–40	1900.3	0.095	0.033	0.510	1.000
		Subsoil b	40–80	1900.4	0.051	0.034	0.740	0.450
N. E. 10 of N. W. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Bluestem grass pasture, broken 1 year corn. Osage loamy silt loam.	Soil	0–7	1902.1	0.192	0.050	0.710	2.380
		Subsurface	7–20	1902.2	0.127	0.036	0.610	1.600
		Subsoil a	20–40	1902.3	0.067	0.038	0.570	0.680
		Subsoil b	40–80	1902.4	0.061	0.037	0.560	0.860
S. W. 10 of N. E. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Corn and wheat 40 years. Osage loamy silt loam.	Soil	0–7	1901.1	0.133	0.042	0.730	1.800
		Subsurface	7–20	1901.2	0.129	0.049	0.600	1.500
		Subsoil a	20–40	1901.3	0.109	0.040	0.570	1.490
		Subsoil b	40–50	1901.4	0.052	0.035	0.550	0.560
S. E. 10 of N. E. 40 of S. E. ¼ sec. 14. Twp. 19 S. R 7., E. of 6 p.m.	Bluestem meadow. Osage silty clay loam. This soil was recently flooded and covered with 6″ silt.	Soil	0–7	1903.1	0.201	0.058	0.780	2.480
		Subsurface	7–20	1903.2	0.122	0.051	0.720	1.450
		Subsoil a	20–40	1903.3	0.070	0.036	0.790	0.930
		Subsoil b	40–90	1903.4	0.038	0.039	0.810	0.530

TABLE 1—Continued

Location, county and land survey	Description, treatment, character and type of soil	Stratum sampled (inches)	Laboratory number	Nitrogen per cent	Phosphorus per cent	Calcium per cent	Organic carbon per cent	Inorganic carbon per cent
Harvey County								
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2 W. of 6 p.m.	Wheat and corn about 14 years then alfalfa 11 years. Dark gray fine sandy loam.	Soil 0–7	1890.1	0.161	0.041	0.570	1.910	0.050
		Subsurface 7–20	1890.2	0.087	0.032	0.450	0.720	
		Subsoil a 20–40	1890.3	0.045	0.023	0.400	0.410	
		Subsoil b 40–80	1890.4	0.036	0.055	0.300	0.160	
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Native bluestem pasture. Fine sandy loam. To be compared with 1890.	Soil 0–7	1891.1	0.189	0.056	0.470	2.280	
		Subsurface 7–20	1891.2	0.101	0.033	0.350	1.010	
		Subsoil a 20–40	1891.3	0.049	0.026	0.460	0.400	
		Subsoil b 40–80	1891.4	0.029	0.026	0.400	0.060	
S. W. 10 of N. W. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Wheat and corn 25 years. Fine sandy loam. To be compared with 1890.	Soil 0–7	1892.1	0.140	0.053	0.600	1.120	
		Subsurface 7–20	1892.2	0.107	0.045	0.670	0.980	
		Subsoil a 20–40	1892.3	0.072	0.043	0.560	0.490	
		Subsoil b 40–80	1892.4	0.056	0.032	0.560	0.410	
S. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Corn and wheat 30 years, then alfalfa 14 years. Heavy silt loam.	Soil 0–7	1893.1	0.200	0.043	0.530	2.310	0.060
		Subsurface 7–20	1893.2	0.097	0.034	0.720	1.630	
		Subsoil 20–36	1893.3	0.075	0.029	1.300	0.800	
N. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Native pasture. Heavy silt loam.	Soil 0–7	1894.1	0.222	0.045	0.620	2.660	0.030
		Subsurface 7–20	1894.2	0.122	0.047	0.740	1.370	
		Subsoil 20–40	1894.3	0.057	0.046	1.030	0.790	
S. E. 10 of the N. W. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Farmed to corn and wheat. Heavy silt loam.	Soil 0–7	1895.1	0.124	0.036	0.570	1.230	0.070
		Subsurface 7–20	1895.2	0.099	0.036	0.680	0.980	
		Subsoil 20–40	1895.3	0.062	0.048	0.990	0.500	

Dickinson County			Sample					
S. E. 10 of S. E. 40 of N. W. ¼ sec. 30. Twp. 12 S. R. 4. E. of 6 p.m.	Alfalfa 15 to 20 years. Osage silt loam.	Soil 0-7	1874.1	0.168	0.048	0.730	1.820	0.0090
		Subsurface 7-20	1874.2	0.117	0.045	0.780	1.200	0.0059
		Subsoil a 20-40	1874.3	0.084	0.047	0.980	0.854	0.2000
		Subsoil b 40-80	1874.4	0.051	0.050	1.960	0.350	
N. E. 10 of S. E. 40 of S. E. ¼ sec. 19. Twp. 12 S. R. 4. E. of 6 p.m.	Native bluestem. Osage loam. To be compared with 1874.	Soil 0-7	1876.1	0.204	0.048	0.940	2.460	0.0059
		Subsurface 7-20	1876.2	0.134	0.042	0.760	1.610	
		Subsoil a 20-40	1876.3	0.109	0.041	0.880	1.120	
		Subsoil b 40-80	1876.4	0.062	0.042	1.020	0.360	
S. W. 10 of S. W. 40 of N. W. ¼ sec. 29. Twp. 12 S. R. 4. E. of 6 p.m.	Farmed to grains, mostly corn for 33 years. Osage silt loam. To be compared with 1874.	Soil 0-7	1875.1	0.140	0.057	1.060	1.820	0.0200
		Subsurface 7-20	1875.2	0.101	0.047	1.070	1.070	0.0046
		Subsoil a 20-40	1875.3	0.084	0.062	1.570	0.960	0.0600
		Subsoil b 40-80	1875.4	0.062	0.063	2.000	0.940	0.2600
N. E. 10 of N. W. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Alfalfa 15-20 years. Brown silt loam.	Soil 0-7	1877.1	0.179	0.044	0.880	2.000	0.0046
		Subsurface 7-20	1877.2	0.134	0.037	0.980	1.260	0.0400
		Subsoil a 20-40	1877.3	0.077	0.040	0.940	0.795	
		Subsoil b 40-80	1877.4	0.060	0.044	1.060	0.320	
N. W. 10 of N. E. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Native pasture. Dark brown silt loam. To be compared with 1877.	Soil 0-7	1879.1	0.204	0.061	0.890	2.630	0.0400
		Subsurface 7-20	1879.2	0.131	0.043	0.890	1.430	
		Subsoil 20-40	1879.3	0.080	0.040	1.040	0.750	
		Subsoil 40-80	1879.4	0.066	0.046	0.930	0.680	
S. E. 10 of N. W. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Corn, wheat, oats for 40 years. Brown silt loam. To be compared with 1877.	Soil 0-7	1878.1	0.163	0.048	0.970	2.006	0.0036
		Subsurface 7-20	1878.2	0.120	0.046	1.010	1.185	0.0046
		Subsoil a 20-40	1878.3	0.070	0.041	1.220	0.640	0.0400
		Subsoil b 40-80	1878.4	0.069	0.041	0.910	0.380	0.0068

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENT				
				Nitrogen per cent	Phosphorus per cent	Calcium per cent	Organic carbon per cent	Inorganic carbon per cent
Harvey County								
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2 W. of 6 p.m.	Wheat and corn about 14 years then alfalfa 11 years. Dark gray fine sandy loam.	Soil 0-7	1890.1	0.161	0.041	0.570	1.910	
		Subsurface 7-20	1890.2	0.087	0.032	0.450	0.720	0.050
		Subsoil a 20-40	1890.3	0.045	0.023	0.400	0.410	
		Subsoil b 40-80	1890.4	0.036	0.055	0.300	0.160	
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Native bluestem pasture. Fine sandy loam. To be compared with 1890.	Soil 0-7	1891.1	0.189	0.056	0.470	2.280	
		Subsurface 7-20	1891.2	0.101	0.033	0.350	1.010	
		Subsoil a 20-40	1891.3	0.049	0.026	0.460	0.400	
		Subsoil b 40-80	1891.4	0.029	0.026	0.400	0.060	
S. W. 10 of N. W. 40 of N.W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Wheat and corn 25 years. Fine sandy loam. To be compared with 1890.	Soil 0-7	1892.1	0.140	0.053	0.600	1.120	
		Subsurface 7-20	1892.2	0.107	0.045	0.670	0.980	
		Subsoil a 20-40	1892.3	0.072	0.043	0.560	0.490	
		Subsoil b 40-80	1892.4	0.056	0.032	0.560	0.410	
S. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Corn and wheat 30 years, then alfalfa 14 years. Heavy silt loam.	Soil 0-7	1893.1	0.200	0.043	0.530	2.310	
		Subsurface 7-20	1893.2	0.097	0.034	0.720	1.630	0.060
		Subsoil 20-36	1893.3	0.075	0.029	1.300	0.800	
N. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Native pasture. Heavy silt loam.	Soil 0-7	1894.1	0.222	0.045	0.620	2.660	
		Subsurface 7-20	1894.2	0.122	0.047	0.740	1.370	0.030
		Subsoil 20-40	1894.3	0.057	0.046	1.030	0.790	

TABLE 1

Analysis of soils in virgin sod, fields cropped to alfalfa and of fields cropped to grain in Kansas

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED		LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
			inches		Nitrogen	Phosphorus	Calcium	Organic carbon	Inorganic carbon
					per cent	per cent	per cent	per cent	per cent
Leavenworth County		Humid section							
N. W. 10 of N. W. 40 of N. E. ¼ Sec. 35. T. 10 S. R. 22 E. of 6 p. m.	Alfalfa 8 years. Blue Grass 10 years before alfalfa. Brown silt loam.	Soil	0–7	1316.1	0.163	0.043	0.530	1.610	
		Subsurface	7–20	1316.2	0.094	0.046	0.480	0.920	
		Subsoil	20–40	1316.3	0.061	0.049	0.640	0.360	
		Subsoil	40–80	1316.4	0.040	0.035	0.610	0.190	0.050
N. E. 10 of S. W. 40 of S. E. ¼ sec. 26. Twp. 10 S. R. 22 E. of 6 p.m.	Cropped to wheat. Well farmed. Brown silt loam.	Soil	0–7	1317.1	0.174	0.048	0.520	1.870	
		Subsurface	7–20	1317.2	0.139	0.041	0.500	1.280	
		Subsoil	20–40	1317.3	0.084	0.034	0.610	0.100	0.070
		Subsoil	40–80	1317.4	0.052	0.044	0.680	0.070	0.040
S. W. 10 of N. E. 40 of N. E. ¼ sec. 15. Twp. 9 S. R. 21 E. of 6 p.m.	Alfalfa 14 years. Before that corn and wheat. Brown silt loam.	Soil	0–7	1318.1	0.222	0.052	0.580	2.500	
		Subsurface	7–20	1318.2	0.177	0.043	0.480	2.180	
		Subsoil	20–40	1318.3	0.121	0.074	0.590	1.640	
		Subsoil	40–80	1318.4	lost				
		Subsoil	80–120	1318.5	0.059	0.042	0.420	0.120	
S. E. 10 of N. E. 40 of N. E. ¼ sec. 5. Twp. 9 S. R. 21 E. of 6 p.m.	Native meadow. Brown silt loam.	Soil	0–7	1319.1	0.296	0.061	0.630	3.760	
		Subsurface	7–20	1319.2	0.225	0.057	0.600	2.540	
		Subsoil	20–40	1319.3	0.144	0.056	0.630	1.120	
		Subsoil	40–80	1319.4	0.082	0.062	0.740	0.260	0.100
		Subsoil	80–100	1319.5	0.085	0.076	0.540	0.200	0.100

Location	Treatment	Depth	Sample					
Brown County								
N. E. 10 of N. W. 40 of S. E. ¼ sec. 34. Twp. 1 N. R. 15 E. of 6 p.m.	Alfalfa 28 years. Marshall silt loam.	Soil 0–7	1768.1	0.211	0.063	0.770	2.280	0.770
		Subsurface 7–20	1768.2	0.142	0.055	0.680	1.540	0.680
		Subsoil 20–40	1768.3	0.062	0.059	0.760	0.400	0.760
		Subsoil 40–80	1768.4	0.031	0.066	0.810	0.060	0.810
S. E. 10 of S. E. 40 of S. E. ¼ sec. 28. Twp. 1 N. R. 15 E. of 6 p.m.	White clover and blue grass. Marshall silt loam.	Soil 0–7	1770.1	0.228	0.061	0.620	2.860	
		Subsurface 7–20	1770.2	0.133	0.051	0.610	1.450	
		Subsoil 20–40	1770.3	0.073	0.051	0.710	0.680	
		Subsoil 40–80	1770.4	0.047	0.049	0.710	0.310	
S. W. 10 of S. E. 40 of N. W. ¼ sec. 34. Twp. 1. N. R. 15. E. of 6 p.m.	Continuous grain cropping. Marshall silt loam.	Soil 0–7	1769.1	0.160	0.048	0.610	1.940	
		Subsurface 7–20	1769.2	0.135	0.048	0.660	1.510	
		Subsoil 20–40	1769.3	0.066	0.053	0.710	0.610	
		Subsoil 40–80	1769.4	0.031	0.066	0.860	0.096	
Nemaha County								
N. W. 10 of S. E. 40 of S. E. ¼ sec. 8. Twp. 5 S. R. 14. E. of 6 p.m.	Alfalfa 21 years. Brown silt loam.	Soil 0–7	1765.1	0.170	0.039	0.660	1.690	0.280
		Subsurface 7–20	1765.2	0.080	0.031	0.700	0.900	
		Subsoil 20–40	1765.3	0.032	0.049	2.000	0.230	
		Subsoil 40–80	1765.4	0.008	0.061	5.280	1.490	
N. W. 10 of S. E. 40 of S. E. ¼ sec. 8. Twp. 5 S. R. 14. E. of 6 p.m.	Cultivated about 45 yrs. Brown silt loam to be compared with 1765.	Soil 0–7	1766.1	0.102	0.040	0.860	1.130	0.030
		Subsurface 7–20	1766.2	0.055	0.044	2.890	0.980	0.620
		Subsoil 20–40	1766.3	0.024	0.050	7.400	1.350	0.960
		Subsoil 40–80	1766.4	0.005	0.059	6.400	0.050	1.650
S. E. 10 of N. E. 40 of N. E. ¼ sec. 17. Twp. 5 E. R. 14. E. of 6 p.m.	Virgin pasture land. Some white clover and blue grass. Brown silt loam.	Soil 0–7	1767.1	0.181	0.038	0.840	1.970	0.050
		Subsurface 7–20	1767.2	0.085	0.042	1.860	0.900	0.240
		Subsoil 20–40	1767.3	0.028	0.049	7.000	0.460	1.940
		Subsoil 40–80	1767.4	0.008	0.058	6.810	0.280	1.680

TABLE 1—Continued

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)		LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
					Nitrogen per cent	Phosphorus per cent	Calcium per cent	Organic carbon per cent	Inorganic carbon per cent
Montgomery County									
S. E. 10 of S. W. 40 of N. W. ¼ sec. 1. Twp. 33 S. R. 14 E. of 6 p.m.	Alfalfa 13 years. Oswego silt loam.	Soil	0–7	1294.1	0.131	0.036	0.429	2.070	
		Subsurface	7–20	1294.2	0.106	0.022	0.485	1.350	
		Subsoil a	20–40	1294.3	0.054	0.029	0.506	0.800	
		Subsoil b	40–80	1294.4	0.031	0.032	0.712	0.520	0.020
		Subsoil c	80–120	1294.5	0.045	0.051	0.774	0.400	0.030
N. E. 10 of N. W. 40 of S. E. ¼ sec. 1. Twp. 33 S. R. 1. E. of 6 p.m.	Native meadow. Oswego silt loam. To be compared with 1294. This land lies a little lower than 1294 and 1295.	Soil	0–7	1296.1	0.186	0.046	0.580	2.590	
		Subsurface	7–20	1296.2	0.114	0.032	0.630	1.640	
		Subsoil a	20–40	1296.3	0.067	0.037	0.530	0.910	
		Subsoil b	40–72	1296.4	0.024	0.056	0.130	0.350	0.160
S. E. 10 of S. W. 40 of N. W. ¼ sec. 1. Twp. 33 S. R. 14. E. of 6 p.m.	Kaffir, corn, and grain for about 35 years. Oswego silt loam. To be compared with 1294.	Soil	0–7	1295.1	0.110	0.034	0.510	1.730	
		Subsurface	7–20	1295.2	0.094	0.028	0.570	1.250	
		Subsoil a	20–40	1295.3	0.050	0.025	0.640	0.710	0.010
		Subsoil b	40–80	1295.4	0.039	0.022	1.010	0.320	0.110
		Subsoil c	80–120	1295.5	0.049	0.055	0.750	0.260	0.030
S. E. 10 of N. E. 40 of S. E. ¼ sec. 13. Twp. 33 S. R. 16. E. of 6 p.m.	Alfalfa 10 years. Oswego silt loam.	Soil	0–7	1297.1	0.168	0.043	1.410	2.320	0.160
		Subsurface	7–20	1297.2	0.087	0.034	1.190	1.150	0.070
		Subsoil a	20–40	1297.3	0.038	0.023	2.170	0.700	0.430
		Subsoil b	40–72	1297.4	0.028	0.027	1.740	0.460	0.310
S. E. 10 of N. E. 40 of S. E. ¼ sec. 13. Twp. 33 S. R. 16. E. of 6 p.m.	General grain and corn farming. Oswego silt loam. To be compared with 1297.	Soil	0–7	1298.1	0.135	0.038	1.050	2.050	0.060
		Subsurface	7–20	1298.2	0.076	0.016	1.930	1.130	0.290
		Subsoil a	20–40	1298.3	0.043	0.026	2.460	0.700	0.500
		Subsoil b	40–72	1298.4	0.026	0.028	1.330	0.410	0.120

Butler County

Location	Description	Layer	Depth	Sample					
S. E. 10 of S. E. 40 of S. E. ¼ sec. 10. Twp. 25 S. R. 5. E. of 6 p.m.	Corn for over 32 years. Osage silt loam. To be compared with 1897.	Soil	0–7	1896.1	0.139	0.027	0.490	1.540	
		Subsurface	7–20	1896.2	0.117	0.031	0.480	1.240	
		Subsoil a	20–40	1896.3	0.087	0.031	0.500	0.920	
		Subsoil b	40–80	1896.4	0.057	0.032	0.500	0.580	
S. E. 10 of N. E. 40 of S. E. ¼ sec. 10. Twp. 25 S. R. 5. E. of 6 p.m.	Native pasture. Osage silt loam. To be compared with 1897.	Soil	0–7	1898.1	0.196	0.035	0.580	2.350	
		Subsurface	7–20	1898.2	0.132	0.035	0.530	1.400	
		Subsoil a	20–40	1898.3	0.077	0.029	0.540	0.890	
		Subsoil b	40–80	1898.4	0.067	0.026	0.550	0.690	
N. E. 10 of S. E. 40 of S. E. ¼ S. E. sec. 10. Twp. 25. R. 5. E. of 6 p.m.	Corn over 20 years, then Alfalfa about 12 years. Osage silt loam.	Soil	0–7	1897.1	0.161	0.038	0.500	1.710	
		Subsurface	7–20	1897.2	0.118	0.040	0.460	1.130	
		Subsoil a	20–40	1897.3	0.071	0.028	0.460	1.070	
		Subsoil b	40–50	1897.4	0.070	0.030	0.580	0.630	

Chase County

Location	Description	Layer	Depth	Sample					
N. W. 10 of N. E. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Alfalfa about 25 years. Osage loamy silt loam.	Soil	0–7	1900.1	0.201	0.039	0.840	2.230	
		Subsurface	7–20	1900.2	0.129	0.041	0.660	1.530	
		Subsoil a	20–40	1900.3	0.095	0.033	0.510	1.000	
		Subsoil b	40–80	1900.4	0.051	0.034	0.740	0.450	
N. E. 10 of N. W. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Bluestem grass pasture, broken 1 year corn. Osage loamy silt loam.	Soil	0–7	1902.1	0.192	0.050	0.710	2.380	
		Subsurface	7–20	1902.2	0.127	0.036	0.610	1.600	
		Subsoil a	20–40	1902.3	0.067	0.038	0.570	0.680	
		Subsoil b	40–80	1902.4	0.061	0.037	0.560	0.860	
S. W. 10 of N. E. 40 of S. W. ¼ sec. 14. Twp. 19 S. R. 7. E. of 6 p.m.	Corn and wheat 40 years. Osage loamy silt loam.	Soil	0–7	1901.1	0.133	0.042	0.730	1.800	
		Subsurface	7–20	1901.2	0.129	0.049	0.600	1.500	
		Subsoil a	20–40	1901.3	0.109	0.040	0.570	1.490	
		Subsoil b	40–50	1901.4	0.052	0.035	0.550	0.560	
S. E. 10 of N. E. 40 of S. E. ¼ sec. 14. Twp. 19 S. R 7, E. of 6 p.m.	Bluestem meadow. Osage silty clay loam. This soil was recently flooded and covered with 6" silt.	Soil	0–7	1903.1	0.201	0.058	0.780	2.480	
		Subsurface	7–20	1903.2	0.122	0.051	0.720	1.450	
		Subsoil a	20–40	1903.3	0.070	0.036	0.790	0.930	
		Subsoil b	40–90	1903.4	0.038	0.039	0.810	0.530	0.040

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENT				
				Nitrogen per cent	Phosphorus per cent	Calcium per cent	Organic carbon per cent	Inorganic carbon per cent
Harvey County								
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2 W. of 6 p.m.	Wheat and corn about 14 years then alfalfa 11 years. Dark gray fine sandy loam.	Soil 0-7	1890.1	0.161	0.041	0.570	1.910	
		Subsurface 7-20	1890.2	0.087	0.032	0.450	0.720	
		Subsoil a 20-40	1890.3	0.045	0.023	0.400	0.410	
		Subsoil b 40-80	1890.4	0.036	0.055	0.300	0.160	0.050
S. E. 10 of N. E. 40 of N. W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Native bluestem pasture. Fine sandy loam. To be compared with 1890.	Soil 0-7	1891.1	0.189	0.056	0.470	2.280	
		Subsurface 7-20	1891.2	0.101	0.033	0.350	1.010	
		Subsoil a 20-40	1891.3	0.049	0.026	0.460	0.400	
		Subsoil b 40-80	1891.4	0.029	0.026	0.400	0.060	
S. W. 10 of N. W. 40 of N.W. ¼ sec. 23. Twp. 24 S. R. 2. W. of 6 p.m.	Wheat and corn 25 years. Fine sandy loam. To be compared with 1890.	Soil 0-7	1892.1	0.140	0.053	0.600	1.120	
		Subsurface 7-20	1892.2	0.107	0.045	0.670	0.980	
		Subsoil a 20-40	1892.3	0.072	0.043	0.560	0.490	
		Subsoil b 40-80	1892.4	0.056	0.032	0.560	0.410	
S. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Corn and wheat 30 years, then alfalfa 14 years. Heavy silt loam.	Soil 0-7	1893.1	0.200	0.043	0.530	2.310	
		Subsurface 7-20	1893.2	0.097	0.034	0.720	1.630	
		Subsoil 20-36	1893.3	0.075	0.029	1.300	0.800	0.060
N. W. 10 of S. E. 40 of S. E. ¼ sec. 2. Twp. 22 S. R. 2. W. of 6 p.m.	Native pasture. Heavy silt loam.	Soil 0-7	1894.1	0.222	0.045	0.620	2.660	
		Subsurface 7-20	1894.2	0.122	0.047	0.740	1.370	
		Subsoil 20-40	1894.3	0.057	0.046	1.030	0.790	0.030
S. E. 10 of the N. W. 40 of		Soil 0-7	1895.1	0.124	0.036	0.570	1.230	

Location (Dickinson County)	Description	Layer	Depth	Sample					
S. E. 10 of S. E. 40 of N. W. ¼ sec. 30. Twp. 12 S. R. 4. E. of 6 p.m.	Alfalfa 15 to 20 years. Osage silt loam.	Soil	0–7	1874.1	0.168	0.048	0.730	1.820	0.0090
		Subsurface	7–20	1874.2	0.117	0.045	0.780	1.200	0.0059
		Subsoil a	20–40	1874.3	0.084	0.047	0.980	0.854	0.2000
		Subsoil b	40–80	1874.4	0.051	0.050	1.960	0.350	
N. E. 10 of S. E. 40 of S. E. ¼ sec. 19. Twp. 12 S. R. 4. E. of 6 p.m.	Native bluestem. Osage loam. To be compared with 1874.	Soil	0–7	1876.1	0.204	0.048	0.940	2.460	0.0059
		Subsurface	7–20	1876.2	0.134	0.042	0.760	1.610	
		Subsoil a	20–40	1876.3	0.109	0.041	0.880	1.120	
		Subsoil b	40–80	1876.4	0.062	0.042	1.020	0.360	
S. W. 10 of S. W. 40 of N. W. ¼ sec. 29. Twp. 12 S. R. 4. E. of 6 p.m.	Farmed to grains, mostly corn for 33 years. Osage silt loam. To be compared with 1874.	Soil	0–7	1875.1	0.140	0.057	1.060	1.820	0.0200
		Subsurface	7–20	1875.2	0.101	0.047	1.070	1.070	0.0046
		Subsoil a	20–40	1875.3	0.084	0.062	1.570	0.960	0.0600
		Subsoil b	40–80	1875.4	0.062	0.063	2.000	0.940	0.2600
N. E. 10 of N. W. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Alfalfa 15–20 years. Brown silt loam.	Soil	0–7	1877.1	0.179	0.044	0.880	2.000	0.0046
		Subsurface	7–20	1877.2	0.134	0.037	0.980	1.260	0.0400
		Subsoil a	20–40	1877.3	0.077	0.040	0.940	0.795	
		Subsoil b	40–80	1877.4	0.060	0.044	1.060	0.320	
N. W. 10 of N. E. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Native pasture. Dark brown silt loam. To be compared with 1877.	Soil	0–7	1879.1	0.204	0.061	0.890	2.630	0.0400
		Subsurface	7–20	1879.2	0.131	0.043	0.890	1.430	
		Subsoil	20–40	1879.3	0.080	0.040	1.040	0.750	
		Subsoil	40–80	1879.4	0.066	0.046	0.930	0.680	
S. E. 10 of N. W. 40 of N. E. ¼ sec. 8. Twp. 12 S. R. 4. E. of 6 p.m.	Corn, wheat, oats for 40 years. Brown silt loam. To be compared with 1877.	Soil	0–7	1878.1	0.163	0.048	0.970	2.006	0.0036
		Subsurface	7–20	1878.2	0.120	0.046	1.010	1.185	0.0046
		Subsoil a	20–40	1878.3	0.070	0.041	1.220	0.640	0.0400
		Subsoil b	40–80	1878.4	0.069	0.041	0.910	0.380	0.0068

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Dickinson County—Continued								
S. E. 10 of S. E. ¼ sec. Twp. 14 S. R. 3. E. of 6 p.m.	Alfalfa 25 years. Broken 3 years. Reseeded to alfalfa. Dark gray silty clay loam. This was at times pastured by hogs.	Soil 0-7	1880.1	0.201	0.091	0.890	2.510	
		Subsurface 7-20	1880.2	0.130	0.043	0.970	1.470	
		Subsoil a 20-40	1880.3	0.083	0.037	1.220	0.770	0.0200
		Subsoil b 40-80	1880.4	0.066	0.041	1.170	0.637	0.0027
N. E. 10 of N. E. 40 of N. E. ¼ sec. 24. Twp. 14 S. R. 3. E. of 6 p.m.	Native bluestem pasture. Dark gray, silty clay loam. To be compared with 1880.	Soil 0-7	1881.1	0.209	0.087	0.920	3.030	
		Subsurface 7-20	1881.2	0.136	0.042	0.990	1.490	
		Subsoil 20-40	1881.3	0.081	0.038	1.040	0.830	0.030
		Subsoil 40-80	1881.4	0.050	0.045	1.220	0.260	0.130
S. W. 10 of S. W. 40 of N. W. ¼ sec. 18. Twp. 14 S. R. 4. E. of 6 p.m.	Farmed mostly to wheat, some corn and kafir, for 35 years. Dark gray silty clay loam. To be compared with 1880.	Soil 0-7	1883.1	0.155	0.045	0.890	2.060	
		Subsurface 7-20	1883.2	0.123	0.038	0.860	1.590	
		Subsoil a 20-40	1883.3	0.082	0.040	0.920	0.840	
		Subsoil b 40-80	1883.4	0.061	0.037	0.920	0.500	
S. E. 10 of S. E. 40 of N. E. ¼ sec. 13. Twp. 14 S. R. 3. E. of 6 p.m.	Alfalfa about 5 years. Dark gray, probably Summit silty clay loam.	Soil 0-7	1882.1	0.157	0.052	0.840	2.270	
		Subsurface 7-20	1882.2	0.113	0.051	0.920	1.400	
		Subsoil 20-40	1882.3	0.081	0.048	1.040	0.840	

Location	Description	Horizon	Depth	Sample No.					
N. E. 10 of the S. W. 40 of S. W. ¼ Sec. 26, Twp. 23, R. 33, W. of 6 P. M.	Alfalfa 27 years. Richland silt loam.	Soil	0–7	1303.1	0.200	0.085	3.030	1.930	0.680
		Subsurface	7–20	1303.2	0.113	0.062	1.680	1.030	0.250
		Subsoil a	20–40	1303.3	0.067	0.067	5.500	0.790	0.970
		Subsoil b	40–80	1303.4	0.035	0.061	4.280	0.490	0.920
		Subsoil c	80–120	1303.5	0.015	0.065	3.000	0.160	0.560
N. W. 10 of the N. W. 40 of N. W. ¼ Sec. 36, Twp. 23 S. R. 33 W. of 6 P. M.	Native Buffalo grass. Richland silt loam. Compare with 1303.	Soil	0–7	1302.1	0.135	0.082	0.990	1.680	0.048
		Subsurface	7–20	1302.2	0.086	0.061	2.090	0.670	0.340
		Subsoil a	20–40	1302.3	0.050	0.055	2.900	0.470	0.600
		Subsoil b	40–80	1302.4	0.034	0.084	1.630	0.130	0.160
		Subsoil c	80–120	1302.5	0.028	0.063	1.200	0.050	0.050
N. W. 10 of the N. E. 40 of N. W. ¼ Sec. 11, Twp. 24 S. R. 33 W. of 6 P. M.	Alfalfa 27 years, Laurel sandy loam.	Soil	0–7	1304.1	0.178	0.064	1.070	1.720	0.300
		Subsurface	7–20	1304.2	0.080	0.052	2.930	0.760	
		Subsoil a	20–40	1304.3	0.048	0.036	5.880	0.790	1.240
		Subsoil b	40–80	1304.4	0.020	0.041	2.140	0.540	0.420
S. E. 10 of the S. W. 40 of S. W. ¼ Sec. 12, Twp. 24 S. R. 33 W. of 6 P.M.	Farmed about 27 years. Laurel sandy loam. Compare with 1304.	Soil	0–7	1305.1	0.079	0.053	1.290	1.040	0.710
		Subsurface	7–20	1305.2	0.057	0.048	1.400	0.690	0.200
		Subsoil a	20–40	1305.3	0.039	0.043	3.540	0.750	0.690
		Subsoil b	40–80	1305.4	0.012	0.040	1.750	0.390	0.380
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32, W. of 6 P. M.	Alfalfa for 30 years, Finney sandy loam.	Soil	0–7	1306.1	0.192	0.078	3.170	2.040	0.680
		Subsurface	7–20	1306.2	0.082	0.041	1.520	0.830	0.230
		Subsoil a	20–40	1306.3	0.040	0.060	3.480	0.670	0.630
		Subsoil b	40–72	1306.4	0.010	0.043	1.350	0.100	0.290
N. W. 10 of the S. E. 40 of S. E. ¼ Sec. 7, Twp. 24, R. 32 W. of 6 P. M.	Native Buffalo grass pasture, laurel loam. Compare with 1306.	Soil	0–7	1308.1	0.099	0.068	0.980	0.890	0.070
		Subsurface	7–20	1308.2	0.052	0.052	2.810	0.300	0.620
		Subsoil a	20–40	1308.3	0.048	0.058	5.180	0.370	1.200
		Subsoil b	40–60	1308.4	0.028	0.039	5.400	0.230	1.510
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32 W. of 6 P. M.	Sorghum, kafir and small grain for 30 years. Finney sandy loam. Compare with 1306.	Soil	0–7	1307.1	0.097	0.062	1.310	0.980	0.100
		Subsurface	7–20	1307.2	0.066	0.050	3.280	0.680	0.730
		Subsoil a	20–40	1307.3	0.036	0.063	5.780	0.920	1.320
		Subsoil b	40–72	1307.4	0.010	0.044	0.740	0.020	0.120

TABLE 1—Continued

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Mitchell County—Continued								
N. E. 10 of the N. W. 40 of S. E. ¼ Sec. 6, Twp. 6 S. R. 10. W. of 6 P.M.	Alfalfa 23 years, gray, Lincoln silt loam, bottom land.	Soil 0–7	1779.1	0.160	0.079	1.300	1.260	0.070
		Subsurface 7–20	1779.2	0.068	0.059	3.260	0.830	0.590
		Subsoil a 20–40	1779.3	0.062	0.058	2.610	0.730	0.400
		Subsoil b 40–72	1779.4	0.048	0.066	1.200	0.630	0.080
S. E. 10 of the N. W. 40 of N. W. ¼ Sec. 6, Twp. 6 S. R. 10. W. of 6 P.M.	Native pasture, gray Lincoln silt loam; bottom land. Compare with 1779.	Soil 0–7	1778.1	0.180	0.068	1.030	1.870	0.040
		Subsurface 7–20	1778.2	0.069	0.056	2.180	0.750	0.380
		Subsoil a 20–40	1778.3	0.048	0.066	1.650	0.390	0.130
		Subsoil b 40–80	1778.4	0.026	0.065	2.710	0.260	0.120
S. E. 10 of the N. W. 40 of N. W. ¼ Sec. 6, Twp. 6 S. R .10. W. of 6 P.M.	Corn and wheat 30 years gray Lincoln silt loam, bottom land. Compare with 1778.	Soil 0–7	1777.1	0.129	0.065	1.500	1.420	0.120
		Subsurface 7–20	1777.2	0.058	0.060	3.130	0.670	0.670
		Subsoil a 20–40	1777.3	0.069	0.064	2.830	0.360	0.490
		Subsoil b 40–80	1777.4	0.064	0.064	1.800	0.950	0.150
S. W. 10 of N. W. 40 of N. W. ¼ Sec. 6, Twp. 6 S. R. 10. W. of 6 P.M.	Wheat and corn 30 years, gray on yellow, silt loam, upland.	Soil 0–7	1781.1	0.115	0.057	0.950	1.340	
		Subsurface 7–20	1781.2	0.046	0.071	2.690	1.230	
N. E. 10 of N. E. 40 of S. E. ¼ Sec. 1, Twp. 6 S. R. 11. W. of 6 P.M.	Native pasture yellow on gray, silt loam, upland.	Soil 0–7	1780.1	0.153	0.055	0.950	1.780	
		Subsurface 7–20	1780.2	0.082	0.058	0.950	0.920	

Osborne County

Location	Description	Sample	No.					
N. W. 40 of the S. W. ¼ Sec. 1, Twp. 8 S. R. 13. W. of 6 P.M.	Alfalfa 20 years, dark gray Lincoln silt loam. The soil had been plowed the spring before sampling.	Soil 0–7	1783.1	0.184	0.060	1.100	2.300	0.080
		Subsurface 7–20	1783.2	0.101	0.059	1.400	1.350	0.060
		Subsoil a 20–40	1783.3	0.062	0.060	1.300	0.660	0.090
		Subsoil b 40–80	1783.4	0.039	0.060	1.400	0.360	
S. E. 40 of the S. W. ¼ Sec. 1, Twp. 8 S. R. 13. W. of 6 P.M.	Native pasture, dark gray Lincoln silt loam.	Soil 0–7	1784.1	0.250	0.068	1.100	3.220	0.090
		Subsurface 7–20	1784.2	0.120	0.063	1.250	1.610	0.560
		Subsoil a 20–40	1784.3	0.066	0.066	3.030	0.780	0.560
		Subsoil b 40–80	1784.4	0.038	0.076	2.950	0.510	
N. W. 40 of the N. W. ¼ Sec. 12, Twp. 8 S. R. 13. W. of 6 P.M.	Farmed to corn and wheat 40 years, dark gray Lincoln silt loam.	Soil 0–7	1782.1	0.134	0.067	1.050	1.660	0.120
		Subsurface 7–20	1782.2	0.096	0.066	1.500	1.250	0.840
		Subsoil a 20–40	1782.3	0.050	0.068	3.910	0.530	0.760
		Subsoil b 40–80	1782.4	0.033	0.067	3.810	0.540	
N. E. 40 of the N. E. ¼ Sec. 12, Twp. 8 S. R. 13. W. of 6 P.M.	Native pasture and meadow, type same as 1785.	Soil 0–7	1786.1	0.232	0.106	1.200	3.710	0.070
		Subsurface 7–20	1786.2	0.120	0.071	1.200	1.440	0.058
N. E. 40 of the N. E. ¼ Sec. 13, Twp. 8 S. R. 13. W. of 6 P.M.	Wheat and corn for 35 years, dark gray, heavy upland soil.	Soil 0–7	1785.1	0.211	0.103	1.350	2.650	0.050
		Subsurface 7–20	1785.2	0.149	0.080	1.150	1.730	0.050
N.W. 40 of S.W. ¼ Sec. 9, Twp. 6 S. R. 13. W. of 6 P.M.	Alfalfa 33 years dark gray Lincoln silt loam.	Soil 0–7	1787.1	0.196	0.070	2.200	2.220	0.320
		Subsurface 7–20	1787.2	0.095	0.085	3.510	1.330	0.650
		Subsoil 20–40	1787.3	0.084	0.077	2.100	1.180	0.280
N. W. 40 of the S. W. ¼ Sec. 9, Twp. 6 S. R. 13. W. of 6 P.M.	Native timber, otherwise the same as 1787.	Soil 0–7	1789.1	0.220	0.100	2.030	3.110	0.240
		Subsurface 7–20	1789.2	0.115	0.098	2.780	1.340	0.520
		Subsoil 20–36	1789.3	0.092	0.087	3.830	1.090	0.890
N. E. 40 of S. W. ¼ Sec. 9, Twp. 6 S. R. 13. W. of 6 P.M.	Corn and wheat for 35 years, otherwise same as 1787.	Soil 0–7	1788.1	0.143	0.090	2.330	1.770	0.350
		Subsurface 7–20	1788.2	0.078	0.084	3.580	1.020	0.680
		Subsoil 20–40	1788.3	0.046	0.090	4.290	0.650	0.930

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Barton County								
N. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Alfalfa 15 years before that wheat and corn 15 years, dark gray Greensburg sandy loam.	Soil 0–7	1884.1	0.159	0.054	0.580	1.910	
		Subsurface 7–20	1884.2	0.058	0.054	0.730	0.570	
		Subsoil a 20–40	1884.3	0.037	0.051	1.340	0.480	0.170
		Subsoil b 40–80	1884.4	0.029	0.048	5.900	0.760	1.470
N. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Buffalo grass pasture, dark Greensburg sandy loam.	Soil 0–7	1885.1	0.106	0.052	0.720	1.280	
		Subsurface 7–20	1885.2	0.050	0.033	0.940	0.390	0.040
		Subsoil a 20–40	1885.3	0.028	0.057	0.950	0.200	0.070
		Subsoil b 40–80	1885.4	0.021	0.041	5.580	0.130	1.370
S. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Wheat and corn 30 years, dark gray Greensburg sandy loam.	Soil 0–7	1886.1	0.137	0.047	1.030	1.680	0.030
		Subsurface 7–20	1886.2	0.076	0.052	1.040	0.940	0.030
		Subsoil a 20–40	1886.3	0.038	0.050	4.230	0.660	1.020
		Subsoil b 40–80	1886.4	0.022	0.042	21.500	0.750	5.930
Pawnee County								
S. E. 10 of N. W. 40 of N. W. ¼ Sec. 7, Twp. 22 S. R. 16 W. of 6 P. M.	Alfalfa 28 years, dark gray Arkansas fine sandy loam.	Soil 0–7	1887.1	0.221	0.053	1.200	2.560	0.070
		Subsurface 7–20	1887.2	0.122	0.052	1.640	1.610	0.150
		Subsoil a 20–40	1887.3	0.059	0.059	4.37	1.150	0.990
		Subsoil b 40–80	1887.4	0.017	0.058	3.860	0.420	0.870

Location	Description	Sample No.	Depth					
S. E. 10 of N. W. 40 of N. W. ¼ Sec. 7. Twp. 22 S. R. 16 W. of 6 P. M.	Buffalo pasture, dark gray Arkansas fine sandy loam.	1888.1	Soil 0–7	0.214	0.059	1.000	2.530	
		1888.2	Subsurface 7–20	0.106	0.046	0.910	0.940	
		1888.3	Subsoil a 20–40	0.053	0.053	2.570	0.570	0.280
		1888.4	Subsoil b 40–80	0.014	0.054	2.820	0.280	0.700
S.E. 10 of S. W. 40 of S. W. ¼ Sec. 6, Twp. 22 S. R. 16 W. of 6 P. M.	Wheat, corn and kafir, 20 years, dark gray Arkansas fine sandy loam.	1889.1	Soil 0–7	0.201	0.093	0.950	2.110	0.005
		1889.2	Subsurface 7–20	0.113	0.058	0.980	1.390	0.051
		1889.3	Subsoil a 20–40	0.072	0.055	3.800	0.540	1.020
		1889.4	Subsoil b 40–80	0.060	0.058	4.640	1.010	1.020
Semi-arid section								
Ford County								
−10 of the S. E. 40 of S. W. ¼ Sec. 27, Twp. 26 S. R. 25 W. of 6 P. M.	Alfalfa 30 years, dark brown silt loam.	1310.1	Soil 0–7	0.210	0.082	1.710	2.080	0.260
		1310.2	Subsurface 7–20	0.085	0.058	2.660	0.610	0.550
		1310.3	Subsoil a 20–40	0.069	0.058	2.710	0.330	0.680
		1310.4	Subsoil b 40–80	0.075	0.044	1.970	0.600	0.290
		1310.5	Subsoil c 80–120	0.046	0.055	2.080	0.290	0.370
−10 of the S. E. 40 of S. W. ¼ Sec. 27, Twp. 26 S. R. 25 W. of 6 P. M.	Native sod. Compare with 1310.	1311.1	Soil 0–7	*0.171*	0.079	1.580	*3.600*	0.160
		1311.2	Subsurface 7–20	0.108	0.033	1.930	0.970	0.300
		1311.2	Subsoil a 20–40	0.061	0.043	2.300	0.540	0.500
		1311.4	Subsoil b 40–80	0.073	0.058	1.780	0.770	0.120
		1311.5	Subsoil c 80–120	0.039	0.040	2.440	0.250	0.430
−10 of the S. E. 40 of S. W. ¼ Sec. 27, Twp. 26 S. R. 25 W. of 6 P. M.	Cultivated 30 years, principally forage crops. Compare with 1310.	1312.1	Soil 0–7	0.136	0.075	1.980	1.820	0.280
		1312.2	Subsurface 7–20	0.079	0.056	2.870	0.910	0.560
		1312.3	Subsoil a 20–40	0.057	0.064	2.450	0.860	0.420
		1312.4	Subsoil b 40–80	0.055	0.055	2.410	0.620	0.500
		1312.5	Subsoil c 80–120	0.051	0.052	1.920	0.480	0.340

TABLE 1—Continued

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Ford County—Continued								
N. W. 10 of the N. E. 40 of N. E. ¼ Sec. 36, Twp. 25 S. R. 25 W. of 6 P. M.	Alfalfa 10 years. Richland silt loam.	Soil 0-7	1313.1	0.156	0.082	0.970	1.660	0.400
		Subsurface 7-20	1313.2	0.051	0.030	2.150	0.990	
S. W. 10 of S. E. 40 of S. E. ¼ Sec. 25, S. R. 25 W. of 6 P. M.	Native pasture, dark gray. Compare with 1313.	Soil 0-7	1315.1	0.152	0.043	0.930	1.740	0.060
		Subsurface 7-20	1315.2	0.089			0.690	
N. W. 10 of the N. E. 40 of N. E. ¼ Sec. 36, Twp. 25 S. R 25 W. of 6 P. M.	Cultivated 30 years. Compare with 1313.	Soil 0-7	1314.1	0.118	0.063	0.890	1.480	0.080
		Subsurface 7-20	1314.2	0.085	0.057	1.710	0.650	0.280
Finney County								
N. E. 10 of the N. E. 40 of S. E. ¼ Sec. 9, Twp. 22, S. R. 33 W. of 6 P. M.	Alfalfa, 20 years. Richland silt loam.	Soil 0-7	1299.1	0.168	0.076	0.930	1.610	
		Subsurface 7-20	1299.2	0.085	0.066	1.340	0.698	0.142
		Subsoil a 20-40	1299.3	0.047	0.054	6.030	0.498	1.282
		Subsoil b 40-80	1299.4	0.040	0.062	4.150	0.707	0.913
N. W. 10 of the N. W. 40 of S. W. ¼ Sec. 10, Twp. 22, S. R. 33 W. of 6 P. M.	Native range, Richland silt loam. Compare with 1299.	Soil 0-7	1300.1	0.137	0.077	0.920	1.940	
		Subsurface 7-20	1300.2	0.084	0.063	1.990	0.460	0.380
		Subsoil a 20-40	1300.3	0.038	0.056	5.670	0.300	0.980
		Subsoil b 40-80	1300.4	0.048	0.076	3.660	0.610	0.720
S. W. 10 of the S. W. 40 of S. W. ¼ Sec. 26, Twp. 23 S. R. 33 W. of 6 P. M.	Farmed to wheat, sorghum and kafir, 20 years. Richland silt loam. Compare with 1303.	Soil 0-7	1301.1	0.134	0.070	1.500	1.760	0.160
		Subsurface 7-20	1301.2	0.101	0.073	3.600	0.750	0.830
		Subsoil a 20-40	1301.3	0.058	0.064	5.350	0.540	1.200
		Subsoil b 40-80	1301.4	0.033	0.089	3.500	0.370	0.840
		Subsoil c 80-120	1301.5	0.028	0.076	2.730	0.430	0.530

Location	Land	Sample	No.	—	—	—	—	—
23, R. 33, W. of 6 P. M.	land silt loam.	Subsoil b 40–80	1303.4	0.035	0.061	4.280	0.490	0.920
		Subsoil c 80–120	1303.5	0.015	0.065	3.000	0.160	0.560
N. W. 10 of the N. W. 40 of N. W. ¼ Sec. 36, Twp. 23 S. R. 33 W. of 6 P. M.	Native Buffalo grass. Richland silt loam. Compare with 1303.	Soil 0–7	1302.1	0.135	0.082	0.990	1.680	0.048
		Subsurface 7–20	1302.2	0.086	0.061	2.090	0.670	0.340
		Subsoil a 20–40	1302.3	0.050	0.055	2.900	0.470	0.600
		Subsoil b 40–80	1302.4	0.034	0.084	1.630	0.130	0.160
		Subsoil c 80–120	1302.5	0.028	0.063	1.200	0.050	0.050
N. W. 10 of the N. E. 40 of N. W. ¼ Sec. 11, Twp. 24 S.R. 33 W. of 6 P. M.	Alfalfa 27 years, Laurel sandy loam.	Soil 0–7	1304.1	0.178	0.064	1.070	1.720	0.300
		Subsurface 7–20	1304.2	0.080	0.052	2.930	0.760	1.240
		Subsoil a 20–40	1304.3	0.048	0.036	5.880	0.790	0.420
		Subsoil b 40–80	1304.4	0.020	0.041	2.140	0.540	
S. E. 10 of the S. W. 40 of S. W. ¼ Sec. 12, Twp. 24 S. R. 33 W. of 6 P.M.	Farmed about 27 years. Laurel sandy loam. Compare with 1304.	Soil 0–7	1305.1	0.079	0.053	1.290	1.040	0.710
		Subsurface 7–20	1305.2	0.057	0.048	1.400	0.690	0.200
		Subsoil a 20–40	1305.3	0.039	0.043	3.540	0.750	0.690
		Subsoil b 40–80	1305.4	0.012	0.040	1.750	0.390	0.380
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32, W. of 6 P. M.	Alfalfa for 30 years, Finney sandy loam.	Soil 0–7	1306.1	0.192	0.078	3.170	2.040	0.680
		Subsurface 7–20	1306.2	0.082	0.041	1.520	0.830	0.230
		Subsoil a 20–40	1306.3	0.040	0.060	3.480	0.670	0.630
		Subsoil b 40–72	1306.4	0.010	0.043	1.350	0.100	0.290
N. W. 10 of the S. E. 40 of S. E. ¼ Sec. 7, Twp. 24, R. 32 W. of 6 P. M.	Native Buffalo grass pasture, laurel loam. Compare with 1306.	Soil 0–7	1308.1	0.099	0.068	0.980	0.890	0.070
		Subsurface 7–20	1308.2	0.052	0.052	2.810	0.300	0.620
		Subsoil a 20–40	1308.3	0.048	0.058	5.180	0.370	1.200
		Subsoil b 40–60	1308.4	0.028	0.039	5.400	0.230	1.510
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32 W. of 6 P. M.	Sorghum, kafir and small grain for 30 years. Finney sandy loam. Compare with 1306.	Soil 0–7	1307.1	0.097	0.062	1.310	0.980	0.100
		Subsurface 7–20	1307.2	0.066	0.050	3.280	0.680	0.730
		Subsoil a 20–40	1307.3	0.036	0.063	5.780	0.920	1.320
		Subsoil b 40–72	1307.4	0.010	0.044	0.740	0.020	0.120

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
		inches		Nitrogen	Phosphorus	Calcium	Organic carbon	Inorganic carbon
				per cent	*per cent*	*per cent*	*per cent*	*per cent*
Barton County								
N. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Alfalfa 15 years before that wheat and corn 15 years, dark gray Greensburg sandy loam.	Soil 0–7	1884.1	0.159	0.054	0.580	1.910	
		Subsurface 7–20	1884.2	0.058	0.054	0.730	0.570	
		Subsoil a 20–40	1884.3	0.037	0.051	1.340	0.480	0.170
		Subsoil b 40–80	1884.4	0.029	0.048	5.900	0.760	1.470
N. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Buffalo grass pasture, dark Greensburg sandy loam.	Soil 0–7	1885.1	0.106	0.052	0.720	1.280	0.040
		Subsurface 7–20	1885.2	0.050	0.033	0.940	0.390	0.070
		Subsoil a 20–40	1885.3	0.028	0.057	0.950	0.200	1.370
		Subsoil b 40–80	1885.4	0.021	0.041	5.580	0.130	
S. E. 10 of S. W. 40 of N. E. ¼ Sec. 17, Twp. 20 S. R. 13 W. of 6 P. M.	Wheat and corn 30 years, dark gray Greensburg sandy loam.	Soil 0–7	1886.1	0.137	0.047	1.030	1.680	0.030
		Subsurface 7–20	1886.2	0.076	0.052	1.040	0.940	0.030
		Subsoil a 20–40	1886.3	0.038	0.050	4.230	0.660	1.020
		Subsoil b 40–80	1886.4	0.022	0.042	21.500	0.750	5.930
Pawnee County								
S. E. 10 of N. W. 40 of N. W. ¼ Sec. 7, Twp. 22 S. R. 16 W. of 6 P. M.	Alfalfa 28 years, dark gray Arkansas fine sandy loam.	Soil 0–7	1887.1	0.221	0.053	1.200	2.560	0.070
		Subsurface 7–20	1887.2	0.122	0.052	1.640	1.610	0.150
		Subsoil a 20–40	1887.3	0.059	0.059	4.37	1.150	0.990
		Subsoil b 40–80	1887.4	0.017	0.058	3.860	0.420	0.870

Location	Description	Sample	No.					
−10 of N. E. 40 of S. W. ¼ Sec. 10. Twp. 12 S. R. 30, W. of 6 P.M.	Alfalfa 15 years, loam bottom land.	Soil 0–7	1802.1	0.133	0.098	2.080	1.440	0.240
		Subsurface 7–20	1802.2	0.067	0.095	2.150	0.570	0.290
		Subsoil a 20–40	1802.3	0.045	0.090	2.730	0.300	0.700
		Subsoil b 40–72	1802.4	0.039	0.078	4.810	0.280	1.170
−10 of the N. E. 40 of S. W. ¼ Sec. 10. Twp. 12 S. R. 30 W. of 6 P.M.	Native meadow loam bottom land. Compare with 1802.	Soil 0–7	1803.1	0.116	0.080	2.130	1.270	0.290
		Subsurface 7–20	1803.2	0.050	0.090	2.450	0.540	0.430
		Subsoil a 20–40	1803.3	0.043	0.088	2.730	0.410	0.560
		Subsoil b 40–72	1803.4	0.033	0.085	3.830	0.270	0.830
S. W. 10 of the S. W. 40 of N. W. ¼ Sec. Twp. 11 S. R. 28 W. of 6 P.M.	Farmed 30 years, mostly wheat, Colby silt loam. Compare with 1805.	Soil 0–7	1804.1	0.118	0.099	1.050	1.310	0.160
		Subsurface 7–20	1804.2	0.075	0.079	1.030	0.810	
S. E. 10 of the S. E. 40 of N. E. ¼ Sec. 7. Twp. 11 S. R. 28, W. of 6 P.M	Native Buffalo grass, Colby silt loam.	Soil 0–7	1805.1	0.170	0.079	1.200	2.060	
		Subsurface 7–20	1805.2	0.080	0.084	1.180	0.600	
S. E. 10 of the N. W. 40 of N.W. ¼ Sec. 18, Twp. 12 S. R. 27, W. of 6 P.M.	Alfalfa 20 years, then cane 3 years, loam bottom land.	Soil 0–7	1800.1	0.184	0.093	3.180	1.900	0.610
		Subsurface 7–20	1800.2	0.079	0.084	4.210	0.920	0.870
Sheridan County								
S. E. 10 of the S. E. 40 of the N. W. ¼ Sec. 28, Twp. 10 S. R. 28 W. of 6 P.M.	Alfalfa 20 years, loam bottom land.	Soil 0–7	1806.1	0.187	0.093	1.350	1.830	0.030
		Subsurface 7–20	1806.2	0.107	0.090	1.450	1.220	0.030
		Subsoil a 20–40	1806.3	0.057	0.082	2.150	0.380	0.030
		Subsoil b 40–80	1806.4	0.043	0.082	4.880	0.360	1.230
S. W. 10 of the S. W. 40 of N. W. ¼ Sec. 28. Twp. 10 S. R. 28 W. of 6 P.M.	Native meadow, loam bottom land. Compare with 1806.	Soil 0–7	1807.1	0.182	0.064	1.200	2.270	
		Subsurface 7–20	1807.2	0.115	0.087	1.200	1.200	
		Subsoil a 20–40	1807.3	0.064	0.097	2.300	0.700	0.310
		Subsoil b 40–72	1807.4	0.033	0.087	3.660	0.320	0.900

TABLE 1—*Continued*

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)		LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
					Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Sheridan County—Continued									
N.W. 10 of the N.W. 40 of N.W. ¼ Sec. 30. Twp. 10 S.R. 28, W. of 6 P.M.	Cultivated 20 years, creek bottom land. Compare with 1806.	Soil	0–7	1808.1	0.118	0.082	1.330	1.140	0.030
		Subsurface	7–20	1808.2	0.058	0.069	2.400	0.460	0.360
		Subsoil a	20–40	1808.3	0.034	0.085	3.530	0.160	0.720
		Subsoil b	40–72	1808.4	0.022	0.079	3.180	0.130	0.680
N. E. ¼ Sec. 6. Twp. 9 S.R. 28, W. of 6 P.M.	Alfalfa 20 years, Colby silt loam.	Soil	0–7	1809.1	0.153	0.084	1.680	1.630	0.110
		Subsurface	7–20	1809.2	0.066	0.084	4.060	0.660	1.260
		Subsoil a	20–40	1809.3	0.040	0.077	3.880	0.340	0.890
		Subsoil b	40–72	1809.4	0.026	0.082	3.430	0.160	0.720
N. E. ¼ Sec. 6. Twp. 9 S. R. 28 W. of 6 P.M.	Native meadow, Colby silt loam. Compare with 1809.	Soil	0.7	1810.1	0.157	0.098	1.200	1.820	0.540
		Subsurface	7–20	1810.2	0.072	0.084	3.180	0.620	0.610
		Subsoil a	20–40	1810.3	0.038	0.086	3.180	0.170	1.250
		Subsoil b	40–72	1810.4	0.035	0.076	5.050	0.080	
Wallace County									
Sec. 35 Twp. 13 S. R. 39, W. of 6 P.M.	Alfalfa 25 years, bottom land.	Soil	0–7	1811.1	0.182	0.099	2.470	1.760	0.280
		Subsurface	7–20	1811.2	0.039	0.063	3.970	0.660	0.400
		Subsoil	20–40	1811.3	0.101	0.073·	2.800	0.390	0.840
Sec. 35. Twp. 13 S. R. 39 W of 6 P.M.	Native pasture, loam bottom land. Compare with 1811.	Soil	0–7	1812.1	0.151	0.080	2.750	1.890	0.400
		Subsurface	7–20	1812.2	0.088	0.070	3.000	1.110	0.210
		Subsoil	20–40	1812.3	0.052	0.055	3.130	0.470	0.600

Location	Description	Depth	No.					
N. E. 10 of the S. W. 40 of S. W. ¼ Sec. 26, Twp. 23, R. 33, W. of 6 P. M.	Alfalfa 27 years. Richland silt loam.	Soil 0–7	1303.1	0.200	0.085	3.030	1.930	0.680
		Subsurface 7–20	1303.2	0.113	0.062	1.680	1.030	0.250
		Subsoil a 20–40	1303.3	0.067	0.067	5.500	0.790	0.970
		Subsoil b 40–80	1303.4	0.035	0.061	4.280	0.490	0.920
		Subsoil c 80–120	1303.5	0.015	0.065	3.000	0.160	0.560
N. W. 10 of the N. W. 40 of N. W. ¼ Sec. 36, Twp. 23 S. R. 33 W. of 6 P. M.	Native Buffalo grass. Richland silt loam. Compare with 1303.	Soil 0–7	1302.1	0.135	0.082	0.990	1.680	0.048
		Subsurface 7–20	1302.2	0.086	0.061	2.090	0.670	0.340
		Subsoil a 20–40	1302.3	0.050	0.055	2.900	0.470	0.600
		Subsoil b 40–80	1302.4	0.034	0.084	1.630	0.130	0.160
		Subsoil c 80–120	1302.5	0.028	0.063	1.200	0.050	0.050
N. W. 10 of the N. E. 40 of N. W. ¼ Sec. 11, Twp. 24 S. R. 33 W. of 6 P. M.	Alfalfa 27 years, Laurel sandy loam.	Soil 0–7	1304.1	0.178	0.064	1.070	1.720	0.300
		Subsurface 7–20	1304.2	0.080	0.052	2.930	0.760	1.240
		Subsoil a 20–40	1304.3	0.048	0.036	5.880	0.790	0.420
		Subsoil b 40–80	1304.4	0.020	0.041	2.140	0.540	
S. E. 10 of the S. W. 40 of S. W. ¼ Sec. 12, Twp. 24 S. R. 33 W. of 6 P. M.	Farmed about 27 years. Laurel sandy loam. Compare with 1304.	Soil 0–7	1305.1	0.079	0.053	1.290	1.040	0.710
		Subsurface 7–20	1305.2	0.057	0.048	1.400	0.690	0.200
		Subsoil a 20–40	1305.3	0.039	0.043	3.540	0.750	0.690
		Subsoil b 40–80	1305.4	0.012	0.040	1.750	0.390	0.380
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32, W. of 6 P. M.	Alfalfa for 30 years, Finney sandy loam.	Soil 0–7	1306.1	0.192	0.078	3.170	2.040	0.680
		Subsurface 7–20	1306.2	0.082	0.041	1.520	0.830	0.230
		Subsoil a 20–40	1306.3	0.040	0.060	3.480	0.670	0.630
		Subsoil b 40–72	1306.4	0.010	0.043	1.350	0.100	0.290
N. W. 10 of the S. E. 40 of S. E. ¼ Sec. 7, Twp. 24, R. 32 W. of 6 P. M.	Native Buffalo grass pasture, laurel loam. Compare with 1306.	Soil 0–7	1308.1	0.099	0.068	0.980	0.890	0.070
		Subsurface 7–20	1308.2	0.052	0.052	2.810	0.300	0.620
		Subsoil a 20–40	1308.3	0.048	0.058	5.180	0.370	1.200
		Subsoil b 40–60	1308.4	0.028	0.039	5.400	0.230	1.510
N. W. 10 of the N. W. 40 of S. E. ¼ Sec. 7, Twp. 24 S. R. 32 W. of 6 P. M.	Sorghum, kafir and small grain for 30 years. Finney sandy loam. Compare with 1306.	Soil 0–7	1307.1	0.097	0.062	1.310	0.980	0.100
		Subsurface 7–20	1307.2	0.066	0.050	3.280	0.680	0.730
		Subsoil a 20–40	1307.3	0.036	0.063	5.780	0.920	1.320
		Subsoil b 40–72	1307.4	0.010	0.044	0.740	0.020	0.120

TABLE 1—Continued

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED (inches)	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen (per cent)	Phosphorus (per cent)	Calcium (per cent)	Organic carbon (per cent)	Inorganic carbon (per cent)
Finney County—Continued								
S. E. 10 of the N. E. 40 of the S.E. ¼ Sec.15. Twp. 24 S. R. 33. W. of 6 P.M.	Alfalfa for 15 years, dark brown, very shallow, Laurel sandy loam.	Soil 0-7	1309.1	0.166	0.075	2.350	1.600	0.450
		Subsurface 7-20	1309.2	0.066	0.049	2.480	0.540	0.670
		Subsoil 20-36	1309.3	0.028	0.045	2.450	0.120	0.580
Gore County								
N. E. ¼ Sec. 29. Twp. 11, R. 26	Native pasture, Grama and Buffalo grass, Colby silt loam.	Soil 0-7	1797.1	0.154	0.084	1.080	1.840	
		Subsurface 7-20	1797.2	0.077	0.094	1.050	0.750	
N. E. ¼ Sec. 29. Twp. 11, R. 26.	Farmed 30 years, mostly wheat, Colby silt loam. Compare with 1797.	Soil 0-7	1796.1	0.128	0.105	1.000	1.350	
		Subsurface 7-20	1796.2	0.070	0.082	1.300	0.670	
N. W. 10 of the S. E. 40 of N. E. ¼ Sec. 12, Twp. 13 S. R. 28 W. of 6 P.M.	Buffalo grass, then alfalfa 15 years, loam bottom land.	Soil 0-7	1798.1	0.210	0.100	1.830	2.230	0.100
		Subsurface 7-20	1798.2	0.086	0.078	2.200	0.900	0.300
		Subsoil a 20-40	1798.3	0.039	0.082	3.560	0.400	0.830
		Subsoil b 40-72	1798.4	0.021	0.097	3.810	0.130	0.860
S. W. 10 of the S. E. 40 of N. E. ¼ Sec. 12, Twp. 13, S. R. 28, W. of 6 P.M.	Native grass, loam bottom land. Compare with 1798. This land was burned over occasionally.	Soil 0-7	1799.1	0.188	0.086	2.000	2.160	0.200
		Subsurface 7-20	1799.2	0.056	0.084	3.110	0.650	0.550
		Subsoil a 20-40	1799.3	0.036	0.077	3.110	0.300	0.670
		Subsoil b 40-72	1799.4	0.015	0.069	3.980	0.330	0.890
N. W. 10 of N. W. 40 of S. E. ¼ Sec. 13. Twp. 3 S. R. 28, W. of 6 P.M.	Grain 15 years, loam bottom land. Compare with 1798.	Soil 0-7	1801.1	0.121	0.090	1.750	1.210	0.120
		Subsurface 7-20	1801.2	0.063	0.086	3.780	0.590	0.800
		Subsoil a 20-40	1801.3	0.030	0.086	4.210	0.180	0.970
		Subsoil b 40-72	1801.4	0.020	0.084	3.060	0.090	0.590

Location	Treatment	Layer	Sample No.					
-10 of N. E. 40 of S. W. ¼ Sec. 10. Twp. 12 S. R. 30, W. of 6 P.M.	Alfalfa 15 years, loam bottom land.	Soil 0–7	1802.1	0.133	0.098	2.080	1.440	0.240
		Subsurface 7–20	1802.2	0.067	0.095	2.150	0.570	0.290
		Subsoil a 20–40	1802.3	0.045	0.090	2.730	0.300	0.700
		Subsoil b 40–72	1802.4	0.039	0.078	4.810	0.280	1.170
-10 of the N. E. 40 of S. W. ¼ Sec. 10. Twp. 12 S. R. 30 W. of 6 P.M.	Native meadow loam bottom land. Compare with 1802.	Soil 0–7	1803.1	0.116	0.080	2.130	1.270	0.290
		Subsurface 7–20	1803.2	0.050	0.090	2.450	0.540	0.430
		Subsoil a 20–40	1803.3	0.043	0.088	2.730	0.410	0.560
		Subsoil b 40–72	1803.4	0.033	0.085	3.830	0.270	0.830
S. W. 10 of the S. W. 40 of N. W. ¼ Sec. 11 S. R. 28 W. of 6 P.M.	Farmed 30 years, mostly wheat, Colby silt loam. Compare with 1805.	Soil 0–7	1804.1	0.118	0.099	1.050	1.310	0.160
		Subsurface 7–20	1804.2	0.075	0.079	1.030	0.810	
S. E. 10 of the S. E. 40 of N. E. ¼ Sec. 7. Twp. 11 S. R. 28, W. of 6 P.M	Native Buffalo grass, Colby silt loam.	Soil 0–7	1805.1	0.170	0.079	1.200	2.060	
		Subsurface 7–20	1805.2	0.080	0.084	1.180	0.600	
S. E. 10 of the N. W. 40 of N. W. ¼ Sec. 18, Twp. 12 S. R. 27, W. of 6 P.M.	Alfalfa 20 years, then cane 3 years, loam bottom land.	Soil 0–7	1800.1	0.184	0.093	3.180	1.900	0.610
		Subsurface 7–20	1800.2	0.079	0.084	4.210	0.920	0.870
Sheridan County								
S. E. 10 of the S. E. 40 of the N. W. ¼ Sec. 28, Twp. 10 S. R. 28 W. of 6 P.M.	Alfalfa 20 years, loam bottom land.	Soil 0–7	1806.1	0.187	0.093	1.350	1.830	0.030
		Subsurface 7–20	1806.2	0.107	0.090	1.450	1.220	0.030
		Subsoil a 20–40	1806.3	0.057	0.082	2.150	0.380	0.030
		Subsoil b 40–80	1806.4	0.043	0.082	4.880	0.360	1.230
S. W. 10 of the S. W. 40 of N. W. ¼ Sec. 28. Twp. 10 S. R. 28 W. of 6 P.M.	Native meadow, loam bottom land. Compare with 1806.	Soil 0–7	1807.1	0.182	0.064	1.200	2.270	0.310
		Subsurface 7–20	1807.2	0.115	0.087	1.200	1.200	
		Subsoil a 20–40	1807.3	0.064	0.097	2.300	0.700	
		Subsoil b 40–72	1807.4	0.033	0.087	3.660	0.320	0.900

TABLE 1—Continued

LOCATION, COUNTY AND LAND SURVEY	DESCRIPTION, TREATMENT, CHARACTER AND TYPE OF SOIL	STRATUM SAMPLED	LABORATORY NUMBER	PLANT-FOOD ELEMENTS				
				Nitrogen	Phosphorus	Calcium	Organic carbon	Inorganic carbon
		inches		per cent	per cent	per cent	per cent	per cent
Sheridan County—Continued								
N. W. 10 of the N. W. 40 of N. W. ¼ Sec. 30. Twp. 10 S. R. 28, W. of 6 P.M.	Cultivated 20 years, creek bottom land. Compare with 1806.	Soil 0–7	1808.1	0.118	0.082	1.330	1.140	0.030
		Subsurface 7–20	1808.2	0.058	0.069	2.400	0.460	0.360
		Subsoil a 20–40	1808.3	0.034	0.085	3.530	0.160	0.720
		Subsoil b 40–72	1808.4	0.022	0.079	3.180	0.130	0.680
N. E. ¼ Sec. 6. Twp. 9 S. R. 28, W. of 6 P.M.	Alfalfa 20 years, Colby silt loam.	Soil 0–7	1809.1	0.153	0.084	1.680	1.630	0.110
		Subsurface 7–20	1809.2	0.066	0.084	4.060	0.660	1.260
		Subsoil a 20–40	1809.3	0.040	0.077	3.880	0.340	0.890
		Subsoil b 40–72	1809.4	0.026	0.082	3.430	0.160	0.720
N. E. ¼ Sec. 6. Twp. 9 S. R. 28 W. of 6 P.M.	Native meadow, Colby silt loam. Compare with 1809.	Soil 0.7	1810.1	0.157	0.098	1.200	1.820	0.540
		Subsurface 7–20	1810.2	0.072	0.084	3.180	0.620	0.610
		Subsoil a 20–40	1810.3	0.038	0.086	3.180	0.170	1.250
		Subsoil b 40–72	1810.4	0.035	0.076	5.050	0.080	
Wallace County								
Sec. 35 Twp. 13 S. R. 39, W. of 6 P.M.	Alfalfa 25 years, bottom land.	Soil 0–7	1811.1	0.182	0.099	2.470	1.760	0.280
		Subsurface 7–20	1811.2	0.039	0.063	3.970	0.660	0.400
		Subsoil 20–40	1811.3	0.101	0.073	2.800	0.390	0.840
Sec. 35. Twp. 13 S. R. 39 W of 6 P.M.	Native pasture, loam bottom land. Compare with 1811.	Soil 0–7	1812.1	0.080	0.080	2.750	1.890	0.400
		Subsurface 7–20	1812.2	0.088	0.070	3.000	1.110	0.210
		Subsoil 20–40	1812.3	0.052	0.055	3.130	0.470	0.600

Trego County

S. W. 10 of the S. W. 40 of S. W. ¼ Sec. 20. Twp. 12 S. R. 23 W. of 6 P.M.	Alfalfa over 20 years, creek bottom silt loam.	Soil 0–7	1794.1	0.131	0.072	1.180	1.060	0.053
		Subsurface 7–20	1794.2	0.052	0.095	2.430	0.660	0.340
		Subsoil a 20–40	1794.3	0.019	0.085	2.560	0.210	0.470
		Subsoil b 40–72	1794.4	0.017	0.084	2.200	0.290	0.230
N. E. 10 of the S. W. 40 of S. W. ¼ Sec. 20. Twp. 12 S. R. 23 W. of 6 P.M.	Wheat prevailing crop, creek bottom land near higher ground, loam or silt loam. Compare with 1794.	Soil 0–7	1795.1	0.160	0.073	1.050	1.840	
		Subsurface 7–20	1795.2	0.076	0.073	1.200	0.800	
		Subsoil a 20–40	1795.3	0.040	0.088	1.100	0.450	
		Subsoil b 40–72	1795.4	0.022	0.099	1.900	0.430	

Fig. 1. Outline Map of Kansas, Showing the Approximate Location Where the Samples Were Taken

problem. A ton of average alfalfa hay contains about 5 pounds of phosphorus. If a soil analyzes 0.05 per. cent of phosphorus, the amount to the depth of 40 inches is 6,000 pounds per acre. Calculating an average yield of 4 tons per year, the amount of phosphorus removed from the soil in 20 years is 400 pounds or a little over 6 per cent of this total. This is quite likely no greater than the difference in the composition of adjoining fields when they were in virgin sod.

The foregoing review shows that when land is in a wild or virgin state, the supply of nitrogen is kept constant or increases; but as soon as the land is cultivated there is a tendency for the nitrogen content to decrease, and that not all of this decrease can be attributed to the removal of nitrogen by the crop. It is also shown that legumes tend to increase the nitrogen content of the soil.

EXPERIMENTAL

The general plan of the experiment reported in this paper was to sample old alfalfa fields, old cultivated fields near by, and virgin sod wherever they could be found adjoining or in close proximity separated by a fence or road. The samples were generally taken in four different strata, namely 0 to 7 inches, 7 to 20 inches, 20 to 40 inches and 40 to 80 inches in depth. These are called surface, subsurface and first and second subsoil, respectively. For the upper three strata a soil augur was generally used. For the lowest it was found best to use a soil tube, particularly in Western Kansas. Borings were made in a number of places in each field. In Western Kansas most of the fields were on bottom land.

The soil samples were put in bags and shipped to the laboratory. When thoroughly air-dry, the whole sample was put in a ball mill and given a preliminary grinding. This served to mix the sample thoroughly. A small subsample was then ground in an ore sample grinder to pass a 100-mesh sieve.

The samples were analyzed for nitrogen, organic carbon, inorganic carbon, phosphorus and calcium. The results of the analyses are given in table 1. The location of the field as given in the first column is by county and by legal description. The location is also indicated in figure 1. In a few cases one field was compared with another across the road and in another county. In such cases the location does not exactly correspond to the county under which it is classed.

There is given in the table a brief description of the soil and its treatment as far as known. For purposes of comparative study, the samples have been divided into three classes according to the location in the state and the amount of rainfall. The samples taken from locations where rainfall is 30 inches or more are classed as from the humid section; those taken where the annual rainfall is between 22 and 30 inches as from the sub-humid, and those where the rainfall is less than 22, as from the semi-arid portions of the state.

TABLE 2
Effect of cropping on the nitrogen content of the surface 7 inches of soil

COUNTY	NITROGEN: POUNDS PER ACRE			DIFFERENCE					
	Native	Alfalfa	Cropped	Alfalfa vs. native		Cropped vs. native		Alfalfa vs. cropped	
				lbs.	per cent	lbs.	per cent	lbs.	per cent
Humid section									
Leavenworth............		3260	3480					−220	6.75
Leavenworth............	5920	4440		−1480	25.00				
Brown.................	4560	4220	3200	+340	7.46	1360	29.83	+1020	24.17
Nemaha...............	3620	3400	2040	−220	6.07	−1580	43.64	+1360	40.00
Montgomery...........	3720	2620	2200	−1100	29.56	1520	40.70	+400	16.04
Montgomery...........		3360	2700					+660	19.64
Butler.................	3920	3220	2780	−700	17.85	−1140	29.10	+440	13.66
Chase.................	3840	4020	2660	+180	4.68	−1180	30.73	+1360	33.83
Harvey................	3780	3220	2800	−560	14.81	−980	25.92	+420	13.03
Harvey................	4440	4000	2480	−440	9.90	−1960	44.14	+1520	38.00
Dickinson.............	4080	3360	2800	−720	17.64	−1280	31.37	+560	16.66
Dickinson.............	4080	3580	3260	−500	12.25	−820	20.09	+320	8.94
Dickinson.............	4180	4020	3100	−160	3.81	−1080	25.83	+920	22.88
Average	4195	3592	2793	−549	13.55	−1290	32.13	+827	22.31
Sub-humid section									
Mitchell...............		4080	3600					+480	11.76
Mitchell...............	4760	5380		+620	13.02				
Mitchell...............	5800		3720			−2080	35.86		
Mitchell...............	3600	3200	2580	−400	11.11	−1020	28.33	+620	19.37
Mitchell...............	3060		2300			−760	24.83		
Osborne...............	5000	3680	2680	−1320	26.40	−2320	46.40	+1000	27.19
Osborne...............	4640		4220			−420	9.05		
Osborne...............	4400	3920	2860	−480	10.90	−1540	35.00	+1060	27.04
Barton................	2120	3180	2740	+1060	50.00	+620	29.44	+440	13.83
Pawnee...............	4280	4420	4020	+140	3.27	−260	6.07	+400	9.05
Average...............	4184	3980	3191	−204	4.87	−993	23.75	+789	18.04
Semi-arid section									
Ford..................	3420	4200	2720	+780	22.80	−700	20.49	+1480	35.23
Ford..................	3040	3120	2360	+80	2.63	−680	22.36	+760	24.35
Finney................	2740	3360		+620	22.62				
Finney................	2700	4000	2680	+1300	48.14	−20	0.74	+1320	33.00
Finney................		3560	1580					+1980	55.62
Finney................	1980	3840	1940	+1860	93.93	−40	2.02	+1900	49.47
Trego.................		2620	3200					−580	22.13
Gove..................	3080		2560			−520	16.88		
Gove..................	3760	4200	2420	+440	11.70	−1340	35.63	+1780	42.38
Gove..................	2320	2600		+340	14.67				
Gove..................	3400		2360			−1040	30.58		
Sheridan..............	3640	3740	2360	+100	2.74	−1280	34.06	+1380	36.89
Sheridan..............	3140	3060		−80	2.55				
Wallace...............	3020	3640		+620	20.54				
Average...........	3020	3495	2418	+606	24.23	−702	20.48	+1077	37.38

THE EFFECT OF CROPPING ON THE NITROGEN CONTENT OF THE SOIL

The number of pounds of nitrogen per acre in 7 inches of the surface soil has been presented in table 2. The differences for virgin soils and for soils cropped in different ways are also given. Soil in native sod is compared with soil cropped to alfalfa, and also with soil which has grown crops other than legumes. A comparison is also made between soil cropped to alfalfa and soils that have grown crops other than legumes. In the first case the native sod is used as the basis of comparison and the second case the soil that had been cropped to alfalfa. The comparison is made both in pounds per acre and on a percentage basis. In calculating the number of pounds per acre, the following weights of soil are assumed:

pounds

Surface, 0–7 inches... 2,000,000
Subsurface, 7–20 inches.. 4,000,000
First subsoil, 20–40 inches..................................... 6,000,000
Second subsoil, 40–80 inches................................... 12,000,000

The results are summarized in table 3.

TABLE 3

Effect of cropping on the nitrogen content of surface soil: Summary

	POUNDS OF NITROGEN PER ACRE		
	Native	Alfalfa	Cropped
Humid...................................	4195	3592	2793
Sub-humid..............................	4184	3980	3191
Semi-arid...............................	3020	3495	2418

In the humid section the cropped soils contained on the average 1402 pounds less nitrogen per acre than the native soils. This means a loss of nearly one-third of the original amount of nitrogen. The soils which had been in alfalfa contained 799 pounds more nitrogen than soils which had grown other crops than legumes, but 603 pounds less than the native soils. This is a loss of 14.3 per cent as compared with the native soils but an increase of 21.6 per cent as compared with the cropped soil.

In the sub-humid section the cropped soils contained 993 pounds less nitrogen than the soils in native sod. This is a loss of nearly one-fourth. The soils in alfalfa contained 789 pounds more nitrogen than the cropped soils, but 204 pounds less than the soils in native sod, a loss in this case of only 5 per cent as compared with the native sod and a gain of 20 per cent as compared with the cropped soil.

In the semi-arid section the cropped soils contained 602 pounds less nitrogen than the soils in native sod, or a loss of one-fifth of the original nitrogen. The alfalfa soils contained 475 pounds of nitrogen more than the native soils, and 1077 pounds more than the cropped soils.

All the fields in alfalfa but one have more nitrogen than those in native sod. In every cropped field but one, the nitrogen content was less than in the native sod. In other words, the fields in alfalfa have over 30 per cent more nitrogen than the cropped soils, and 15.7 per cent more than the soils in native sod. This represents an unmistakable gain of nitrogen through the growing of alfalfa. It should be borne in mind that the average age of the alfalfa fields in the sub-humid and semi-arid section was much greater than that of the fields in the humid section.

It is probable that the decrease in nitrogen of the fields in alfalfa in the humid and sub-humid sections took place before the alfalfa was seeded. The increase in nitrogen, if any, since the alfalfa was seeded has not been sufficient to restore the original amount. For reasons to be presented later, it is probable that in some fields at least, the growth of alfalfa has simply prevented a further decrease.

Perhaps more accurate conclusions can be drawn from the data presented in table 4 in which only those fields are included which were close enough together to permit direct comparisons. The figures in the first three columns are the same as those in table 2. In the next two columns are given the number of years of continuous alfalfa or grain production in the respective fields. By subtracting the number of pounds of nitrogen in the continuously cropped field from that in native sod, the loss of nitrogen by cropping is obtained. These figures are given in the sixth column. The average yearly loss obtained by dividing the total by the number of years in cultivation is given in the seventh column.

The average yearly loss multiplied by the number of years the land was in grain crops gives the total loss sustained before seeding to alfalfa. The figures so obtained are given in the eighth column. Subtracting this from the amount present in the native sod gives the amount presumably present in the soil when the alfalfa was seeded. These figures are given in the ninth column. By comparing this amount with that present when the sample was taken the gain or loss through the continuous growing of alfalfa can be calculated. The results of this calculation are given in the last column of table 4.

It will be seen that in both the humid and the sub-humid sections, the gains are practically balanced by the losses. The growing of alfalfa has simply prevented further losses or has secured enough from the air to take the place of that removed by the crop. In the semi-arid section there is an unmistakable gain. This corroborates the statements made on the basis of the figures presented in table 2.

If continuous growing of alfalfa simply maintains a balance in the nitrogen content of the soil, it seems that the amount of nitrogen in the hay crop is about equal to that obtained from the air, while that in the roots and stubble is about equal to that obtained from the soil.

Another factor however, must be considered, viz; the loss of leaves in curing and stacking. The loss is probably greater in the semi-arid section than in the more humid sections. The intense sunshine causes the leaves to fall very easily if the period of drying is prolonged even a few hours. It has been calculated that from 7 to 25 per cent of the leaves are lost in curing and stacking, and from 3 to 14 per cent of the entire crop (9).

TABLE 4

Gain or loss of nitrogen as a result of cropping to alfalfa

COUNTY	POUNDS OF NITROGEN PER ACRE			YEARS IN ALFALFA	YEARS IN A GRAIN CROP	LOSS OF NITROGEN			NITROGEN PER ACRE WHEN SEEDED	GAIN OR LOSS WHILE IN ALFALFA
	Alfalfa	Native	Cropped			Total loss by cropping to grains	Average yearly loss	Loss before seeding to alfalfa		
						lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Humid section										
Brown.........	4220	4560	3200	28	45	1380	31	527	4050	+170
Nemaha........	3420	3620	2040	21	45	1580	35	840	2760	+649
Montgomery.....	2620	3720	2200	12	35	1520	43	989	2730	−110
Butler.........	3220	3920	2780	12	32	140	4	80	3840	+620
Chase.........	4020	3840	2660	25	40	1180	29	435	3405	+615
Harvey........	3220	3780	2800	11	25	980	39	546	3234	−14
Harvey........	4000	4440	2480	14	44	1960	44	1320	3120	+880
Dickinson......	3360	4080	2800	20	35	1280	37	555	3530	−170
Dickinson......	3580	4080	3260	20	40	820	20	400	3680	−100
Dickinson......	4020	4180	3100	25	35	1080	31	310	3870	+150
Sub-humid section										
Mitchell........	3200	3600	2580	23	30	1020	34	238	3370	−170
Osborne........	3680	5000	2680	20	40	2320	58	1160	3840	−160
Osborne........	3920	4400	2860	33	35	1540	44	88	4310	−390
Pawnee........	4420	4280	4020	28	20	260	13	0	4280	+140
Semi-arid section										
Ford...........	4200	3420	2720	30	30	700	23	0	3420	+780
Ford...........	3120	3040	2360	10	30	680	22	220	2580	+540
Finney.........	4000	2700	2680	27	20	20	0	0	2700	+1300
Finney.........	3840	1980	1940	30	30	40	0	0	1980	+1860
Gove..........	4200	3760	2420	15	15	1340	89	0	3760	+440
Sheridan.......	3740	3640	2360	20	20	1280	64	0	3640	+100

We may assume that on the average, 20 per cent of the leaves, or about 10 per cent of the crop, is lost. When bad weather prevails during harvesting, as much as half the crop may be lost. This no doubt is a factor in increasing the nitrogen content of the soil. Assuming an annual yield of hay of 5000 pounds per acre, half of which is leaves, the nitrogen added to the soil in

TABLE 5

Effect of cropping on the organic carbon in the surface 7 inches of soil

COUNTY	POUNDS OF ORGANIC CARBON PER ACRE			DIFFERENCE					
	Native	Alfalfa	Cropped	Alfalfa vs. Native		Cropped vs. Native		Alfalfa vs. Cropped	
				lbs.	per cent	lbs.	per cent	lbs.	per cent
Humid section									
Leavenworth		32,200	37,400					−5,200	16.14
Leavenworth	75,200	50,000		−25,200	33.51				
Brown	57,200	45,600	38,800	−11,600	20.25	−18,400	32.16	+6,800	14.91
Nemaha	39,400	33,800	22,600	−5,600	14.21	−16,800	42.64	+11,200	30.18
Montgomery	51,800	41,400	34,600	−10,400	20.07	−17,200	33.20	+6,800	16.42
Montgomery		46,400	41,000					+5,400	11.63
Butler	47,000	34,200	30,800	−12,800	27.23	−16,200	34.46	+3,400	9.94
Chase	47,600	44,600	36,000	−3,000	6.30	−11,600	24.36	+8,600	19.28
Harvey	45,600	38,200	22,400	−7,400	16.22	−23,200	50.87	+15,800	41.36
Harvey	53,200	46,200	24,600	−7,000	13.16	−28,600	53.75	+21,600	46.75
Dickinson	49,200	36,400	36,400	−12,800	26.01	−12,800	26.01	0	0
Dickinson	52,600	40,000	40,120	−12,600	23.95	−12,480	23.72	−120	00.30
Dickinson	60,600	50,200	41,200	−10,400	17.16	−19,400	32.01	+9,000	17.92
Average	52,673	41,477	33,810	−11,196	21.26	−18,863	35.82	+7,667	18.48
Sub-humid section									
Mitchell	62,800	64,600		+1,800	3.00				
Mitchell		48,400	43,600					+4,800	9.91
Mitchell	57,900		41,800			−16,100	27.80		
Mitchell	37,400	25,200	28,400	−12,200	32.62	−9,000	24.06	−3,200	12.69
Mitchell	35,600		26,800			−8,800	24.71		
Osborne	62,200	44,400	35,400	−17,800	28.61	−26,800	43.08	+9,000	20.27
Osborne	64,400	46,000	33,200	−18,400	25.57	−31,200	48.45	+12,800	27.82
Osborne	74,200		53,000			−21,200	28.57		
Barton	25,600	38,200	33,600	+12,600	49.21	+8,000	31.25	+4,600	12.09
Pawnee	50,600	51,200	42,200	+600	1.18	−8,400	16.60	+9,000	17.57
Average	52,289	45,429	37,556	−6,860	13.12	−14,733	28.19	+7,873	17.33

Semi-arid section

Ford	72,000	41,600	36,400	−30,400	42.22	−35,600	49.44	+5,200	12.50
Ford	34,800	33,200	29,600	−1,600	4.59	−5,200	14.94	+3,600	10.84
Finney	33,600	38,600	35,200	+5,000	14.88	+1,600	4.76	+3,400	8.80
Finney	38,800	32,200		−6,600	17.01				
Finney	17,800	40,800	19,600	+23,000	29.21	+1,800	10.11	+21,200	51.96
Finney		34,400	20,800					+13,600	39.54
Trego		21,200	36,800					−15,600	73.58
Gove	43,200	44,600	24,200	+1,400	3.24	−19,000	43.98	+20,400	45.74
Gove	25,400	28,800		+3,400	13.38				
Gove	36,800		27,000			−9,800	26.63		
Gove	41,200		26,200			−15,000	36.40		
Sheridan	45,400	36,600	22,800	−8,800	19.38	−22,600	49.78	+13,800	37.70
Sheridan	36,400	32,600		−3,800	10.43				
Wallace	37,800	35,200		−2,600	6.87				
Average	38,600	34,983	27,860	−3,846	9.96	−10,740	27.83	+8,200	+23.50

this way would amount to 17.5 pounds annually or a total of 350 pounds in 20 years. There may be two reasons for no apparent gain in the humid and sub-humid sections: (1) The leaves remain on the surface of the soil and the nature of the decay is such that a portion of the nitrogen is lost. (2) Alfalfa uses the nitrogen liberated by decay and nitrification of organic matter. When the nitrogen is abundant more nitrogen is used. In this way the addition of nitrogen through the loss of leaves may create such a condition that less is taken from the air.

The fact must not be lost sight of that alfalfa takes large amounts of nitrogen from the air which is not accounted for in this investigation, because no data on the amount removed in the crops are available. For every ton of hay harvested, it appears that 50 pounds of nitrogen has been taken from the air. With the best methods of farming the greater part of this can be returned to the soil.

Another effect of growing alfalfa is an improved physical condition of the soil, as a result of the large, deeply penetrating roots. Alfalfa roots are large and contain a large amount of organic matter; they decay easily and rapidly making available an abundance of the mineral elements, phosphorus, and potassium contained in the alfalfa roots. The process of decay also liberates more of these elements from the insoluble compounds found in the soil.

Finally it should be remembered that the fields studied were comparatively well stocked with nitrogen when alfalfa was seeded. If the investigation had been made on fields which had lost more nitrogen before the alfalfa was seeded, the results probably would be different.

THE EFFECT OF CROPPING ON THE ORGANIC CARBON CONTENT OF THE SOIL

The organic carbon content of surface soil of fields in native sod, alfalfa and those cropped to grain is given in table 5. The differences are also given in pounds and percentage, the comparisons being made as in table 2. The results are summarized in table 6.

In the humid section the fields cropped to grain contain on the average 18,863 pounds less of organic carbon than those in native sod, an average loss of nearly 36 per cent. The fields in alfalfa contain an average of 7667 pounds more than those in grain crops, but 11,196 pounds less than those in native sod. This is a loss of 21.26 per cent as compared with the native sod and a gain of 18.48 per cent as compared with the cropped fields. All the soils in native sod have more organic carbon than the fields in alfalfa or those cropped to grains. All but two of the fields in alfalfa have more organic carbon than the fields cropped to grains.

In the sub-humid section the fields cropped to grains contain an average of 14,733 pounds less of organic carbon than the native sod. This is a loss of nearly 28 per cent. The fields in alfalfa contained an average of 7873 pounds of organic carbon more than those in grains, but 6860 pounds less

than the fields in native sod. This is a loss of a little over 13 per cent as compared with the native sod and a gain of over 18 per cent as compared with the cropped land.

In the semi-arid section the fields cropped to grain contained 10,740 pounds less organic carbon than those in native sod. This is a loss of nearly 30 per cent. Those in alfalfa contained on the average 7123 pounds more organic carbon than those cropped to grains, but 3817 pounds less than those in native sod, a loss of about 10 per cent as compared with the native sod, and a gain of over 25 per cent as compared with the fields cropped to grains. All but four of the fields in native sod contained more organic carbon than those cropped to alfalfa or grain.

These results show that the growing of alfalfa has not fully restored the organic carbon. There is no doubt as to the possibility of increasing the organic carbon content of the soil by growing alfalfa, but in the majority of cases studied, such cropping has resulted in a further decrease.

We must recognize the possibility of error in the determinations, since we do not know the exact carbon content of these fields, at the time the alfalfa

TABLE 6

Effect of cropping on the organic carbonic content of the surface soil: Summary

	POUNDS PER ACRE		
	Native	Alfalfa	Cropped
Humid............................	52,673	41,477	33,810
Sub-humid........................	50,638	45,429	37,536
Semi-arid........................	38,433	34,983	26,720

was seeded. The approximate amounts can be calculated however, by the same method employed in studying the loss of nitrogen. Data secured by this method are presented in table 7.

The data corroborate those presented in table 5 and the statements made in relation to them. Unlike the effect on the nitrogen there has not been an increase in the carbon content of these soils as a result of cropping to alfalfa. This is true for all the three sections.

THE EFFECT OF CROPPING ON THE NITROGEN AND ORGANIC CARBON CONTENT
OF THE SUBSOIL

An attempt has been made to compare the nitrogen and organic carbon content of the different strata. The results are presented in tables 8 and 9. In interpreting these results it should be remembered that the experimental errors of determination are rather large. There are two reasons for this. Most of these samples are from bottom land. While the fields compared appeared to be very uniform, the subsoils were often found to differ considerably. Also the weight of the subsoil from 20 to 40 inches deep was assumed

TABLE 7

Gain or loss of organic carbon as a result of cropping to alfalfa

COUNTY	POUNDS OF ORGANIC CARBON PER ACRE			YEARS IN ALFALFA	YEARS IN GRAIN CROP	LOSS OF ORGANIC CARBON			POUNDS OF ORGANIC CARBON PER ACRE WHEN SEEDED	GAIN OR LOSS WHILE IN ALFALFA
	Alfalfa	Native	Cropped			Total loss by cropping to grain	Average yearly loss	Loss before seeding to alfalfa		
						lbs.	*lbs.*	*lbs.*		*lbs.*
Humid section										
Brown	45,600	57,200	38,800	28	45	18,400	409	6,953	50,247	−4,647
Nemaha	33,800	39,400	22,600	21	45	16,800	373	8,952	30,448	+3,352
Montgomery	41,400	51,800	34,600	12	35	17,200	491	11,293	40,507	+893
Butler	34,200	47,000	30,800	12	32	16,200	506	10,120	36,880	−2,680
Chase	44,600	47,600	36,000	25	40	11,600	290	4,350	43,250	+1,350
Harvey	38,200	45,600	22,400	11	25	23,200	928	12,992	32,608	+5,592
Harvey	46,200	53,200	24,600	14	44	28,600	650	19,500	33,700	+12,500
Dickinson	36,400	49,200	36,400	20	35	12,800	365	5,475	43,725	−7,325
Dickinson	40,000	52,600	40,120	20	40	12,480	312	6,240	46,360	−6,360
Dickinson	50,200	60,600	41,200	25	35	19,400	554	5,540	55,060	−4,860
Sub-humid section										
Mitchell	25,200	37,400	28,400	23	30	9,000	300	−2,100	35,300	−10,100
Osborne	46,000	64,400	33,200	20	40	31,200	780	15,600	48,800	−1,800
Osborne	44,400	62,200	35,400	33	35	26,800	765	1,530	60,670	−16,270
Pawnee	51,200	50,600	42,200	28	20	8,400	420	0	50,600	+600
Semi-arid section										
Ford	41,600	72,000	36,400	30	30	35,600	1,186	0	72,000	−30,400
Ford	33,200	34,800	29,600	10	30	5,200	173	3,460	31,340	+1,860
Finney	38,600	33,600	35,200	27	20	−1,600*	−80	0	33,600	+5,000
Finney	40,800	17,800	19,600	30	30	−1,800*	−60	----	17,800	+23,000

to be 6,000,000 pounds per acre, and the subsoil from 40 to 80 inches 12,000,000 pounds per acre. When these sums are multiplied by the significant errors in the determinations, the differences between duplicate determination is often greater than the differences due to the cropping.

It will be seen that the method of cropping has not influenced materially the nitrogen content of the subsoil below a depth of 20 inches, except possibly

TABLE 8

Average nitrogen content of the soil of different strata

SECTION	KIND OF SOIL	0-7 INCHES	7-20 INCHES	20-40 INCHES	40-80 INCHES
		per cent	*per cent*	*per cent*	*per cent*
Humid...............	Native	0.200	0.121	0.069	0.045
	Alfalfa	0.175	0.111	0.062	0.043
	Cropped	0.136	0.105	0.068	0.046
Sub-humid.............	Native	0.209	0.101	0.059	0.032
	Alfalfa	0.200	0.092	0.061	0.033
	Cropped	0.159	0.088	0.051	0.036
Semi-arid..............	Native	0.151	0.079	0.048	0.037
	Alfalfa	0.173	0.077	0.051	0.035
	Cropped	0.121	0.073	0.042	0.025

TABLE 9

Average carbon content of the soil of different strata

SECTION	KIND OF SOIL	0-7 INCHES	7-20 INCHES	20-40 INCHES	40-80 INCHES
		per cent	*per cent*	*per cent*	*per cent*
Humid...............	Native	2.52	1.39	0.77	0.44
	Alfalfa	2.09	1.27	0.72	0.48
	Cropped	1.67	1.23	0.81	0.43
Sub-humid.............	Native	2.72	1.16	0.67	0.31
	Alfalfa	2.27	1.14	0.90	0.63
	Cropped	1.88	1.11	0.54	0.69
Semi-arid..............	Native	1.93	0.71	0.41	0.34
	Alfalfa	1.63	0.74	0.46	0.37
	Cropped	1.39	0.70	0.55	0.29

in the semi-arid section. In the subsurface (7 to 20 inches), the nitrogen content of the fields in native sod is slightly higher than the others, the difference being greatest when the comparison is made with the fields cropped to grain. It is sometimes thought that plants secure nitrogen from the subsoil and deposit it in the surface soil. The facts here presented do not support this theory.

TABLE 10

Effect of cropping on the phosphorus content of the surface 7 inches of soil

COUNTY AND SECTION	POUNDS OF PHOSPHORUS PER ACRE			DIFFERENCES					
	Native	Alfalfa	Cropped	Alfalfa vs. native		Cropped vs. native		Alfalfa vs. cropped	
				lbs.	*per cent*	*lbs.*	*per cent*	*lbs.*	*per cent*
Humid									
Leavenworth...........	1220	1040		−180	14.75				
Leavenworth...........		860	960					−100	9.61
Brown................	1220	1260	960	+40	3.27	−260	21.31	+300	23.80
Nemaha..............	760	780	800	+20	2.63	+40	5.26	−20	2.56
Montgomery..........	920	720	680	−200	21.73	−240	26.08	+40	5.55
Montgomery..........		860	760					+100	11.62
Butler...............	700	760	540	+60	8.57	−160	22.85	+220	28.94
Chase................	1000	780	840	−220	22.00	−160	16.00	−60	7.69
Harvey..............	1120	820	1060	−300	26.78	−60	5.35	−240	29.26
Harvey..............	900	860	720	−40	4.44	−180	20.00	+140	16.27
Dickinson............	960	960	1140	0	0	+180	18.75	−180	18.75
Dickinson............	1220	880	960	−340	27.86	−260	21.31	−80	9.09
Dickinson............	1740	1820	900	+80	4.59	−840	48.27	+920	50.54
Average............	1069	954	860	−115	10.76	−209	19.55	+94	9.85
Sub-humid									
Mitchell..............	1260	1380		+120	9.52				
Mitchell..............	1360	1580	1300	+220	16.17	−60	4.41	+280	17.72
Mitchell..............	1100		1140			+40	3.63		
Mitchell..............	2100		1920	−720	34.29	−180	8.57	−540	39.13
Mitchell..............		1500	1440	+240	19.05	+180	14.28	+60	4.00
Osborne..............	2000	1400	1800	−600	30.00	−200	10.00	−400	28.57
Osborne..............	1360	1200	1340	−160	11.76	−20	1.47	−140	11.66
Osborne..............	2120		2060			−60	2.83		
Barton...............	1040	1080	940	+40	3.84	−100	9.61	+140	12.96
Pawnee..............	1180	1060	1860	−120	10.17	+680	57.71		75.47
Average............	1502	1314	1533	−123	16.85	+31	2.06	−219	16.67
Semi-arid									
Ford.................	1580	1640	1500	+60	3.79	−80	5.06	+140	8.53
Ford.................	860	1640	1260	+780	90.61	+400	46.51	+380	23.17
Finney...............	1540	1520		−20	1.29				
Finney...............	1640	1700	1400	+60	3.65	−240	14.63	+300	17.64
Finney...............	1360	1560	1240	+200	14.70	−120	8.82	+320	20.51
Finney...............		1280	1060				.	+220	17.18
Trego................		1440	1460					−20	1.38
Gove................	1720	2000	1800	+280	16.27	+80	46.51	+200	10.00
Gove................	1600	1960		+360	22.50			−600	40.00
Gove................	1680	1500	2100	−180	10.71	+420	25.00		
Gove................	1580		1980			+400	25.31		
Sheridan.............	1280	1860	1640	+580	45.31	+360	28.10	+220	11.82
Sheridan.............	1960	1680		−280	14.28				
Wallace..............	1600	1980		+380	23.75				
Average............	1533	1674	1544	+141	9.19	+152	10.00	+130	8.48

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The organic carbon content of the subsoil below 20 inches appears not to be materially affected by the method of cropping. In two cases the carbon content of the fields cropped to grain is the highest of any. This suggests that the differences are less than the error of determination. There appears to be a significant though slight difference in the subsurface (7 to 20 inches) in favor of the fields in native sod. Also, those in alfalfa appear to be slightly higher in carbon than those cropped to grain. As previously pointed out there are rather large differences in the carbon content of the surface soil (0 to 7 inches).

The fact that the principal loss of carbon is in the surface soil suggests that it is practically all due to the oxidation of organic matter, to soil blowing or to erosion. These factors also affect the nitrogen content.

THE EFFECT OF CROPPING ON THE PHOSPHORUS CONTENT OF THE SOIL

The phosphorus content of the soil is indicated in table 10. The data are arranged in the same way as for the nitrogen and for the organic carbon content presented above. In general, the differences are so small that no definite conclusions can be reached.

A ton of alfalfa hay contains about 5 pounds of phosphorus. At this rate 20 average crops would remove only about 500 pounds. If all of it came from the surface soil the removal of this amount no doubt would be noticeable, but a deep-rooted crop like alfalfa secures phosphorus from considerable depth. The smallest difference which can possibly be significant in a phosphorus determination is 0.005 per cent. This amounts to 600 pounds of phosphorus per acre to a depth of 40 inches assuming the weight of the soil to be 12,000,000 pounds. Accordingly, the amount of phosphorus removed by 20 average crops of alfalfa could not be determined with any degree of certainty. Also, there is no certainty that all fields had the same phosphorus content to begin with.

Considering the possibilities of error it is not probable that the differences in the phosphorus content of the fields cropped in different ways are significant. The rather high phosphorus content of the surface soil of the fields cropped to alfalfa in the semi-arid section is perhaps explained by the loss of leaves in curing.

The phosphorus content of the different strata is presented in table 11. Excepting only the cropped soils in the humid section, the surface averages higher in phosphorus than the subsoils. This accords with what would be expected. The phosphorus in the native vegetation becomes incorporated with the surface soil when the former decays or is burned off.

The fact that the cropped soils in the humid section do not contain more phosphorus than the lower strata may be taken to mean that so much of the phosphorus of the surface soil has been used that the average has been reduced to that found in the lower depths. In the sub-humid and semi-arid sections the soils have not been cultivated for so long a time, therefore this condition has apparently not yet been reached.

THE EFFECT OF CROPPING ON THE CALCIUM CONTENT OF THE SOIL

The principal object in analyzing the soils for calcium was to determine the calcium content of soils where alfalfa grows successfully. The amount of calcium in crops is small in comparison with the amount present in the soil, and with the amounts removed from the soil by leaching. A ton of alfalfa hay contains from 20 to 25 pounds of calcium. The amount in grain crops is much smaller. On the other hand, it is estimated that the equivalent of 500 pounds per acre of $CaCO_3$ is removed by leaching every year in a humid climate. The amount of calcium in these soils varies more than that of any other element.

As a rule the calcium content increases from the surface downward. This is especially true for soils with a high calcium content and for the sub-humid and semi-arid sections. By reference to table 1 it will be seen that most

TABLE 11

Phosphorus, average percentages in different strata

SECTION	KIND OF SOIL	0–7 INCHES	7–20 INCHES	20–40 INCHES	40–80 INCHES
		per cent	*per cent*	*per cent*	*per cent*
Humid section...........	Native	0.053	0.041	0.039	0.039
	Alfalfa	0.046	0.039	0.037	0.044
	Cropped	0.042	0.041	0.042	0.042
Sub-humid..............	Native	0.075	0.063	0.064	0.056
	Alfalfa	0.066	0.062	0.059	0.057
	Cropped	0.075	0.066	0.065	0.057
Semi-arid..............	Native	0.079	0.070	0.064	0.072
	Alfalfa	0.084	0.067	0.067	0.067
	Cropped	0.077	0.068	0.070	0.070

soils in the humid section contain from 0.5 to 1.0 per cent calcium in the surface soil. Of those devoted to alfalfa growing, few contained less than 0.5 per cent of calcium. In the sub-humid and semi-arid sections the percentage of calcium in practically all soils studied is 1 per cent and above. In none of the soils of these two sections can there be said to be a calcium deficiency. It is very probable that those soils which contain 0.5 per cent or less calcium are very near the border-line of calcium deficiency, and especially so when there is no material increase in calcium in the lower strata.

THE EFFECT OF CROPPING ON THE INORGANIC CARBON CONTENT OF THE SOIL

In the humid section only 6 out of 38 soils contained inorganic carbon in the surface or subsurface (table 1). In the subsoil, the occurrence of inorganic carbon was more frequent. This means that in this section calcium carbonate is absent from all but a very few soils and that the carbonate has disap-

peared from the surface and subsurface to a larger extent than from the subsoil. This shows that these soils are rapidly approaching a calcium deficiency. The calcium in forms other than the carbonate are relatively unavailable. In the sub-humid and semi-arid sections, only 11 soils out of 60 have no inorganic carbon in the surface soil, and of these 11, only 5 have no inorganic carbon in the subsoil. This means that practically all soils in the sub-humid and semi-arid sections have not only an abundance of calcium but that a large amount of it is in the carbonate form which is the most available.

SUMMARY AND CONCLUSIONS

1. It is generally assumed that alfalfa helps maintain the fertility of the soil by securing nitrogen from the air and by preventing the oxidation of the humus and organic matter which takes place rapidly when cultivated crops are grown; no exact figures seem to have been secured, however. It is very important that such data be available because when the alfalfa crop is removed it is quite possible that the field is left no richer in nitrogen than if no alfalfa had been grown, and because alfalfa removes a relatively large amount of other plant-food elements.

2. Kansas produces more alfalfa than any other state, and has some of the oldest alfalfa fields in the country, some of which have been continuously in alfalfa for 30 years or more. This offers unusual opportunities: or a study of this kind.

3. The general plan of the experiment was to sample these old alfalfa fields and other fields nearby which were of the same type of soil but had been continuously in cultivation or in native sod. Preference was given to those locations where all these three fields were close enough together for direct comparison. The soil was generally sampled in four depths: 0 to 7 inches, 7 to 20 inches, 20 to 40 inches, and 40 to 80 inches. The samples were analyzed for nitrogen, organic carbon, inorganic carbon, phosphorus and calcium.

4. For purposes of comparisons, the state was divided into three sections; humid, sub-humid and semi-arid. The basis of division was the average annual rainfall. All samples taken from localities where the rainfall is 30 inches or more, were classed as from the humid section; those from localities where the rainfall is between 30 and 22 inches as from the sub-humid section; and those from localities where the rainfall is less than 22 inches as from the semi-arid section.

5. It was found that in the humid section the surface of the cropped soil had lost one-third of the nitrogen as compared with the surface soil of the native sod. The fields in alfalfa contained 14.3 per cent less nitrogen than the native sod, and 21.6 per cent more than those fields which had been cropped to grain. In the subsurface 7 to 20 inches, the changes due to cropping were much less than in the surface, and in the subsoils there were no changes that could be attributed to the methods of cropping.

6. In the sub-humid section the fields cropped to grain lost one-fourth of the nitrogen as compared with the surface soil of the native sod. The alfalfa fields contain 5 per cent less nitrogen than the native sod, but 20 per cent more than the fields in grain. In this section the changes due to cropping are also confined to the surface and subsurface.

7. In the semi-arid section the cropped soil has lost one-fifth of the nitrogen as compared with the native sod. Alfalfa fields contained 15.7 per cent more nitrogen than the soils in native sod, and 30 per cent more than the soils continuously cropped. In this section the significant changes are confined to the surface soil.

8. This comparison shows that there is an unmistakable gain in nitrogen of the soils in the semi-arid section. This may be attributed in part at least to a greater loss of leaves in curing alfalfa in the arid section.

9. It may be concluded that the continuous growing of alfalfa, may not increase the nitrogen content of the soil. The increased crop production which is usually obtained on alfalfa fields when they are plowed up is probably due to the large amount of available nitrogen they contain.

10. In the humid section, the cropped soils have lost 36 per cent of the organic carbon present in the virgin sod and those in alfalfa over 21 per cent. The fields in alfalfa contain 18 per cent more than those continuously cropped to grain.

11. In all sections the changes in the organic carbon content are confined to the surface soil. There is just one exception to this statement. In the humid section, the subsurface of the native sod has a significantly higher carbon content than the alfalfa or cropped soil.

12. In the sub-humid section the cropped soils have lost 28 per cent of the original carbon content and the alfalfa soils 13 per cent.

13. In the semi-arid section, the cropped soils contain 30 per cent less carbon than the virgin soils and the alfalfa soils 10 per cent less. In this section the changes in carbon content differ fundamentally from the changes in nitrogen which, as previously pointed out, show a gain.

14. The phosphorus content of the cropped soil is lower than that of the alfalfa soil, or soil in native sod. Alfalfa removes more phosphorus from the soil than grain crops. The fact that the alfalfa fields do not show a lower phosphorus content than the soils in native sod, may be taken to mean that there has been a transference of phosphorus from the subsoil to the surface. This transference has probably taken place because of falling of leaves. Because of the small per cent of phosphorus and the limits of determinations, this conclusion is not so well substantiated as those in regard to nitrogen and carbon.

15. In the humid section, the surface of the cropped soil contains the same amount of phosphorus as the subsoils. In all other cases, the phosphorus content of the surface soil is higher than that of the subsoils. This would point to a definite lowering of the phosphorus content of the cropped soil in the humid section.

16. In the sub-humid and semi-arid sections, most of the soils contain 1 per cent and more of calcium and nearly all have some calcium in the carbonate form. In the humid section, alfalfa was found growing on four soils successfully in which the calcium content was less than 0.5 per cent. The calcium content in most soils varies from 0.50 to 1 per cent, and in the majority the carbonate form of the calcium is absent.

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ACTIVITY OF SOIL ACIDS

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Technically the activity of an acid is measured by the concentration of hydrogen-ions which it gives. Activity is determined, therefore, by solubility and ionization.

It would be rather presumptive, however, to say that the activity of the acids present is the only factor to consider, in the study of soil reaction. Soils are very complex and may contain a large number of acids and acid salts, as well as a great variety of bases. The interrelationships of the various soil acids and bases is also extremely complex, and very difficult to point out in specific terms.

There are at least two distinct causes which have been given for the acid condition of soils. One theory is that soil acidity is not due to real acids but that it is a physical phenomenon which has been called adsorption. The other theory which is subdivided into two, is that soil acidity is due to actual acids, some holding that these acids are primarily of organic origin but more believing that the acids are mostly of mineral origin.

METHODS

There are standard methods for measuring the activity of acids but most of them are not easily adapted to soils. Truog (11) has proposed a method which depends upon the competition of soil acids with a standard acid (acetic) for base. It has not proved very satisfactory so far as tested in this laboratory. The method used in this work was designed primarily for the determination of the amount of lime taken up by acid soils and is not presented as especially adapted to a determination of acid activity. But it gives some valuable information on this phase of the problem and hence the results obtained are presented in the following pages.

Such degrees of activity as the method detects depend upon the rate of reaction of acid soil and pure calcium carbonate brought together in water suspension and thoroughly mixed by constant shaking. The carbon dioxide is removed as rapidly as evolved, absorbed in sodium hydroxide and titrated at suitable intervals. This is a modification of the Tacke method (10), the details of which are given in a previous discussion of acidity methods.

In general there are two types of acidity methods applied to soil work. By one the concentration of hydrogen-ion is determined. This is active acidity which may cause direct toxicity. The other type determines the amount of base taken up by acid soils. Most methods come in this latter class. Such methods may include in their results all active acids capable of giving a high concentration of acid ion, and also all those acids which may be capable of reacting but give only neutral indications with indicators. A good example of such acids is potassium acid phthalate, which is more properly speaking an acid salt, but nevertheless it reacts with its full equivalent of base. Insoluble acids and proteins might be expected to behave in much the same way, though reacting more slowly.

It would seem that information in regard to acid soils is not complete until both its degree of acidity and its capacity for taking up lime are understood. Unfortunately, no one method combines these two phases of the question in one determination.

TABLE 1

Lime requirement* at intervals of 3, 6, 9 and 21 hours

	3 HOURS	6 HOURS	9 HOURS	21 HOURS	TOTAL
Soil 1					
Loam	5,000 lbs.*	6,100 lbs.*	6,500 lbs.*	7,000 lbs.*	
Increase		1,100 lbs.	400 lbs.	500 lbs.	7,000 lbs.
Per cent of total	71.5	15.8	5.6	7.1	100

* These results are all expressed as pounds per acre, of calcium carbonate

Practically all methods permit the reaction of readily active acids. There is, however, a great variation in their capacity to show a reaction with slowly active acids. This is evidenced by the fact that some methods show two or three times the lime requirement of others. The point of equilibrium and the rapidity with which reactions occur are determined many times not primarily by the activity of the acids but by the more or less violent actions of heat and strong bases and also by the mass law. When one product of the reaction is removed, equilibrium represents a more advanced stage of decomposition. Such hastening of the reaction as occurs from the removal of a product of the reaction is perfectly legitimate. Any more violent stimulation of the reaction would likely lead to results that would never be obtained under natural conditions in the field. The method selected seems more natural and normal than any other method in common use.

EXPERIMENTAL

In this study the rate of reaction of soil acids in different soil has been compared and this in turn compared with the rate of reaction of the acidity of buffered solutions of known hydrogen-ion concentration. None of the

first soils used were high in organic matter so that the acidity is probably mineral rather than organic. Titrations were made at various intervals, the blank on the soil and reagents being subtracted each time so that the results show the relative reactivity of the different fractions of acidity.

The above table shows that nearly three-fourths of the soil acids had re- acted at the end of 3 hours, assuming complete reaction to have taken place in the whole run. During the second 3 hours only a little more than a fifth as much as the first and during the third 3 hours about a third as much as during the second period, reacted. During the last 12 hours only one-tenth as much reacted as during the first 3 hours. These results show that there is a considerable portion of readily reactive acids while there is also an appre- ciable portion that is quite slowly active. Similar results are shown in table 2.

TABLE 2

Lime requirement at intervals of 5, 8 and 20 hours

	5 HOURS	8 HOURS	20 HOURS	TOTAL
Soil 1				
Loam................................	5,660 lbs.	6,480 lbs.	7,020 lbs.	
Increase.............................		820 lbs.	540 lbs.	
Per cent of total.....................	80.6	11.7	7.7	100

In this test four-fifths of the acidity reacted at the end of 5 hours. At the other intervals the results are very similar to those in the first test, being nearly identical during the last 12 hours of the run. Results with soil 2, which was more clayey than the first, are shown in table 3.

TABLE 3

Lime requirement at intervals of 9, 24 and 36 hours

	9 HOURS	24 HOURS	36 HOURS	TOTAL
Soil 2				
Clay loam...........................	4,425 lbs.	5,700 lbs.	6,750 lbs.	
Increase............................		1,275 lbs.	1,050 lbs.	
Per cent of total.....................	65.6	18.9	15.5	100

This soil was run on the basis that nine or ten hours should be long enough to determine all acidity of a degree of activity sufficient to render harmful effects in the soil. One fact is quite noticeable, viz., that acids were less reactive in this soil than in the preceding one. While nearly two-thirds of the acidity reacted at the end of 9 hours, nearly one-fourth as much reacted during the last 12 hours after having run a period of 24 hours. Since this soil was inclined toward a clay texture, it was probably rich in acid silicates and these were evidently slowly reactive. It is probable that during the reaction, hydrolytic processes occurred and perhaps also an exchange of bases, but such reactions have occurred at a progressively decreasing rate.

In table 4 the results of tests of soils low in organic matter and of low lir requirement are given, titrations being made at intervals of 3 hours. Lik wise some soils high in organic matter were subjected to the same runs f comparison.

It will be observed that soil 3 acted as though it had a considerable reser acidity. The lime requirement was fairly high and the last 3 hours of t run gave nearly one-third as much as the first 3 hours. It reacted very d ferently from No. 4 which had a lower lime requirement. The differen may have been due partly to the amount, as well as kinds of acid presei

TABLE 4

Lime requirement at intervals of 3 hours

	TIME			TOTAL
	3 HRS.	6 HRS.	9 HRS.	
Soil 3				
Sandy loam...........................	3,700 lbs.	5,100 lbs.	6,200 lbs.	
Increase.............................		1,400 lbs.	1,100 lbs.	
Per cent of total.....................	59.7	22.6	17.7	100
Soil 4				
Sand...............................'...	800 lbs.	1,100 lbs.	1,100 lbs.	
Increase.............................		300 lbs.	0	
Per cent of tota......................	72.7	27.3	0	100
Soil 5				
Miami silt...........................	1,800 lbs.	2,300 lbs.	2,500 lbs.	
Increase.............................		500 lbs.	200 lbs.	
Per cent of total.....................	72.0	20.0	8.0	
Soil 6				
Muck...............................	15,200 lbs.	19,400 lbs.	25,400 lbs.	
Increase.............................		4,200 lbs.	6,000 lbs.	
Per cent of total.....................	60.0	16.4	23.6	100
Soil 7				
Loam, organic........................	12,400 lbs.	14,200 lbs.	17,000 lbs.	
Increase.............................		1,800 lbs.	2,800 lbs.	
Per cent of total.....................	72.9	10.6	16.5	100

The first soil evidently had a larger total acidity, though the reactive porti was relatively smaller. The more highly ionized acids should depress 1 less active ones and the reaction should continue longer for that reason. T second soil which had a lower lime requirement had a smaller depression ionization of the less active acids, consequently the reaction was nearly co plete sooner. Previous data indicate, however, that several hours may required on some soils before an approximate completion of reaction is reach It is not believed that a reaction would ever be absolutely complete, as hydr ysis or hydration or other changes in time may develop a new acidity.

It was thought that soils 6 and 7, being very acid and rather high in organic matter, would react for a longer time. Consequently, they were run a total of 23 hours. There was a marked distinction between the loam and the muck soil, the latter reacting much more slowly. This again may be explained as due to depression of ionization of the less reactive acids, especially by the buffering of the organic matter of the muck soil.

The sandy type of soil reacted more readily, since there was less buffering. But silicious soils may have a considerable portion of insoluble and unreactive acids, as the data indicate.

To get a comparison of the relative rates of reaction during the early runs the rate per hour is given in table 5. This can be obtained only for equivalent periods, since it would obviously be unfair to compare the rate for a

TABLE 5

Rate per hour of reaction

SOIL NO.	FIRST 3 HOURS	RATE PER HOUR	SECOND 3 HOURS	RATE PER HOUR	THIRD 3 HOURS	RATE PER HOUR	FOURTH 3 HOURS	RATE PER HOUR	17 HOURS	RATE PER HOUR
1	5,000	1,666	1,100	366	400	133	500	42		
3	3,700	1,233	1,400	466	1,100	366				
4	800	266	300	100	0	0				
5	1,800	600	500	166	200	66				
6	15,200	5,066	200	1,400					6,000	353
7	12,400	4,133	1,800	600					2,800	165

Per cent of succeeding hours, based on the first hourly rate

	SECOND 3 HOURS	THIRD 3 HOURS	FOURTH 3 HOURS	17 HOURS
1	21.4	8.0	2.5	
3	37.8	29.6		
4	40.0	0		
5	27.6	11.0		
6	27.6			7.0
7	14.5			4.0

3-hour with a rate for a 10-hour run. Such a comparison gives a standard basis which is not possible in presenting percentages of total acidity, since such totals nearly always depend upon the length of the run.

A very great variation in the rate of reactivity may be observed. The amount of acidity which reacted during the second 3 hours varied all the way from 14.5 to 40 per cent of that which reacted during the first 3 hours. During the third 3 hours the variation was nearly as great, running from 0 to 29.6 per cent of the amount evolved during the first 3 hours. The few figures obtained for the very long runs show that the muck was holding out more persistently than those soils which have less organic matter. This would indicate a comparatively large reserve of acidity for soils high in organic matter. But mineral soils may have a rather variable reserve, as is apparent

in the case of soil 3, which gave a high run during the third 3 hours. Soil 1 may be noted for its low reserve. The data apparently indicate that the rate of reactivity and reserve acidity does not depend entirely on the type of soil.

Some greenhouse studies were made with lime-treated acid soils in gallon jars. The lime requirement, taken from another study, by the modified Tacke method was 2.75 tons. Lime as carbonate was applied at the rate of 1-ton increments up to 10 tons. At the end of 2 weeks the 1-ton treatment was nearly completely exhausted. By 4 weeks 2 tons were practically exhausted, and with the larger applications, 6 to 10 tons, an amount of carbonate practically equivalent to the acidity was taken up at the end of 2 weeks. The average of all high treatments, 6, 7, 8, 9 and 10 tons, was exactly 2.75 tons used by the soil. The determinations were made by obtaining residual carbonates and subtracting from the treatments.

These are significant figures and indicate rapid reaction between acid soil and lime when no artificial hastening is provided other than intimate contact in the presence of moisture. Reaction was not complete at this point, however, as the average amount of lime taken up by these same pots 21 weeks later was 4.62 tons, or nearly 1.7 times as much. For the sake of comparison, however, it may be observed that while reaction was at the rate of nearly 1.4 tons per week during the first two weeks, it was only 0.09 ton per week during the following 21 weeks. After this if determinations had been made, the reaction rate would have been extremely slow.

These results show quite definitely that the active acids of soils are neutralized rather quickly in the presence of base, but that the same soil continues to decompose carbonates very slowly even when there is no leaching. So far as evidence goes this absorption of base might continue indefinitely.

The same soils were likewise tested for acidity both quantitatively and qualitatively at various intervals. The first qualitative test was not applied until the end of 4 weeks, but at this time the 3-ton treated soil gave a neutral test by the Truog lead acetate method. The soils receiving treatments of 2 tons were slightly acid. The quantitative tests gave similar results. This again indicates that the active acidity was quickly neutralized even though the soil continued to take up a considerable quantity of lime. These data are good evidence that a soil may remain quite active in decomposing base after all appreciable acidity is neutralized.

These results are wholly from the side of the acidity present. But the base used has a very marked effect also, as may be readily shown. Thus while precipitated calcium carbonate applied at the rate indicated as the lime requirement by the Tacke method, and thoroughly mixed with the soil which was moistened to the optimum moisture content, caused the soil to react to give a neutral qualitative test by simply standing 24 hours, three times the amount of natural magnesian limestone did not give a neutral indication even after several days. The fineness of division is a factor in the above results, but the nature of the base is likewise a factor.

Under field conditions lack of mixing and intimate contact, as well as the coarseness of the material used, would undoubtedly result in a rather slow reaction, but even then it may be presumed that the more reactive acids are neutralized comparatively rapidly.

STUDIES WITH KNOWN H-ION CONCENTRATION

By way of comparison with the rate of reaction of acid soils, several buffered solutions were prepared and tested as to their ability to decompose calcium carbonate. First, acetic acid was buffered with potassium acetate, using 0.2 N acid and normal acetate. With an H-ion concentration of 2.2×10^{-5} the full capacity of the buffered acid was exhausted in a 3-hour run, the 10 cc. of acid used liberating 10 cc. of carbon dioxide in the titration. Similar results were obtained when the H-ion was 1.3×10^{-6} and 7.6×10^{-6} in all tests made. Thus carbon dioxide was evolved from calcium carbonate with each concentration used, to the full capacity of the acid present. The neutral potassium acetate alone liberated no carbon dioxide, as would be expected. With phenolphthalein present it was possible to follow the reaction by the pink color which developed at the end of 2 hours, indicating that the acid had been exhausted. The experiment ran an hour longer, however, in all cases before titration.

Another test was made with known acid and hydrogen-ion concentration in the presence of the soil. The effect would then be that of the acid added plus that of the soil acids. Two soils were used, one sandy and low in organic matter, the other containing less sand and higher in organic matter. It was found that the soil exercised no appreciable depressing effect upon the reaction between the acid and the carbonate. With a hydrogen-ion concentration as low as 7.6×10^{-6} the full capacity of the acid to liberate carbon dioxide was exhausted in a 3-hour run, and the second 2 hours gave a practically constant acidity with and without the acid added. In other words, after the first 3 hours the reaction proceeded as though no acid had been added to the soil. Both soils behaved in the same way and although one had a rather high lime requirement, this requirement was not depressed and the full capacity of the acid was exhausted in the first 3 hours. The sandy soil alone liberated 1 cc. of carbon dioxide and the soil plus 10 cc. of N/2 acetic acid, buffered to give a hydrogen-ion concentration of 7.6×10^{-6} liberated 10.3 cc. or only 0.7 cc. short of the theoretically possible amount, from both soil and acid.

Another buffer solution prepared from sodium hydroxide and monopotassium acid phosphate (50 cc. of 0.2 M KH_2PO_4 and 29.63 cc. of 0.2 M. NaOH diluted to 200 cc.) with a hydrogen-ion concentration of 1×10^{-7}, or the same as that of pure water, was used. Likewise (using the Clarke and Lubs (3) formulae again) a concentration of H-ion of 1.6×10^{-6} was prepared. The neutral and nearly neutral solutions did not react to a measurable extent as might be expected.

In the same way, using potassium acid phthalate and sodium hydroxide, H-ion concentrations of 6.3×10^{-5}, and 1×10^{-5}, also 1×10^{-6} were tested. The first two of these reacted liberating 10.1 cc. and 4.7 cc., respectively, of carbon dioxide titrated as N/10 acid. The other concentration did not react, and all reactions were complete at the end of 3 hours. It is probable that the reaction was really complete sooner, as was indicated by the color given to the indicater (phenolphthalein) after one to two hours, but titrations were made only at 3-hour intervals. The above determinations were made primarily to discover what degree of buffering would yet permit of reaction between the acid and calcium carbonate.

With the same idea in view an N/10 solution of glycocoll and also some albumin and casein were tested. All failed to decompose the carbonate. It is noticeable in the above cases that potassium acid phthalate which is neutral to methyl orange will react with carbonate, or sodium hydroxide, and may be titrated with phenolphthalein, while the glycocoll and proteins used, fail to react. It would seem that compounds which contain the amino group must be in the form of an "inner salt," as is sometimes suggested, the amino groups neutralizing the free carboxyl groups. As decomposition occurs in the soil the carboxyl groups must be set free, but whether they remain to react with base is doubtful.

Since all of the buffered solutions tested, if capable of reacting at all, react to completion by the end of three hours or less, meaning that no further decomposition occurs during a subsequent run, it would seem that soil acids may be subject to considerable buffering and yet react with lime at an appreciable rate. Insolubility is probably one of the more important causes for the slow reaction. The prolonged decomposition of carbonate by acid soils is undoubtedly due in part to a continuous hydration of silica, or to the hydrolysis of various alumino-silicic acid salts. It cannot be determined at what point such reactions would cease. These results would indicate also that even the more actively functioning portion of acidity of most soils may be held at a low ionic concentration due to buffering. The ionic concentrations which have proved capable of reacting with carbonates are lower than have proved injurious to both plants and soil organisms. But the studies of Sharp and Hoagland and Plummer have shown that acid soils may have a higher concentration of acidity than is compatible with the most efficient functioning of many plants and bacteria. But even in quite acid soils organisms may be active locally if in contact with, or protected by such highly buffered materials as the proteins. It is characteristic of emulsoid colloids to act as protective colloids by forming a thin film about the colloidal particles of another substance. Such protection can operate against an acidity as well as to prevent precipitation of the protected colloid. The organic colloids or protein particles may, therefore, serve as centers of bacterial activity, in much the same way as lime particles, though perhaps less effectively.

HOW DO SOILS BECOME ACID?

With the above experimental data as a basis the question of the origin of acid soils may be taken up. The explanation of the development of an acid condition in soils can probably be given only in part. The question may be conveniently discussed under three topics, mineral acids, organic acids, and colloids.

Mineral acids

If it is assumed that the mineral portion of the earth was once a molten magma, then by cooling, igneous rocks were formed and from these by weathering or metamorphic changes, rocks of secondary classification were derived. In this way, together with the accumulation of organic debris, soils were built up. The original magma being primarily a mixture of oxides, and silicates of alumina, soils are of the same general nature. Igneous rocks run as high as 80 per cent silicates and rarely drop below 40 per cent. Some soils are even richer in silica than this, partly because of the leaching and weathering of less resistant constituents.

The formation of soil from minerals has been predominantly a hydration process, supplemented by oxidations and carbonations. Because of the hydration, marked changes occur, not only in weight and bulk but in reaction and in susceptibility to reaction. During the change not only water but oxygen and carbon dioxide are absorbed. Minerals are converted into acid salts, and these constantly undergo further change by disintegration or metathesis. Hydration processes may produce colloids, of which clay is a good example. When an anhydride becomes hydrous it may assume acid or basic properties, of which clay again is an example. According to Loew pure clay is capable of ionizing as both an acid and a base. But under agricultural conditions the acid radicals are replaced by a base, if not naturally, then by the addition of basic material to the soil.

The most important of the soil-forming silicates are the feldspars and feldspathoids. In fact, feldspars constitute about 60 per cent of igneous rocks, and in tracing changes which occur in their weathering, a suggestion as to the origin of acid soils may be obtained.

Starting with the feldspars, the first change which occurs is probably simple hydration which would result in the formation of zeolites or other hydrates. Long contact with water is the only essential for this change to occur. Thus soda feldspar, albite ($Na_2O.Al_2O_3.6SiO_2$) might change to the zeolite, natrolite ($Na_2O.Al_2O_3.3SiO_2.2H_2O$) by hydration and the splitting off of 3 molecules of silicic anhydride (SiO_2). After hydration further changes probably occur rather rapidly. In fact, it is disputed that zeolites exist in soils. But other hydrated silicates do exist.

As the soil is tilled and bacterial activity is increased, the rate of decay of minerals is much increased. Consequently, cultivated soils are likely to be-

come acid much more rapidly. The soil bases are leached as bicarbonates and the production of carbon dioxide naturally stimulates such changes Thus the loss of lime from the zeolite, chabizite, (CaO.Al$_2$O$_3$.4SiO$_2$.2H$_2$O) would probably follow or accompany the hydration of the original feldspar The calcium would thus leach away as bicarbonate, leaving probably some silica, and clay (Al$_2$O$_3$.2SiO$_2$.2H$_2$O), which, though it probably seldom exist: in the soil in pure form, is nevertheless a complex acid salt.

The above reactions are not given as those which must occur but only as typical of those which are likely to occur. The feldspars and other alumino silicates are really salts of various hypothetical silicic acids which are weak acids, and such bases as potassium, sodium and calcium, which are strong bases. It is entirely logical that such salts should be hydrolyzed, leaving the quite insoluble and slightly ionized acid or acid salt to accumulate in the soil, while the soluble base is leached away. Most bases are rather soluble as bicarbonates, and even the silicates of sodium and potassium are highly leachable.

As specific examples there may be mentioned calcium feldspar, which is a salt of alumino-disilicic acid; potash feldspar, a salt of alumino-hexasilicic acid, natrolite and leucite, salts of alumino-tri and alumino-tetra-silicic acids, respectively. The prefixes di-, tri-, etc., refer to the number of silicic acid molecules which occur in the compound. The di-silicic acid, though it might not be obtained in the free state, would represent the combination of two molecules of silicic acid with an accompanying loss of one molecule of water. As hydrolysis occurs, the acids must be partially or wholly freed and therefore, rendered active and capable of taking up lime or other bases which might come into contact with them. Ash and Ash give a formula (2H$_2$O 2Al$_2$O$_3$.8SiO$_2$) which is an alumino silicic acid and as salts of such an acid (2Na$_2$O.2Al$_2$O3.8SiO$_2$) analcine, or (CaO.Na$_2$O.2Al$_2$O$_3$.8SiO$_2$) andesite. Although the specific formulae are not of great significance they are of value for the sake of illustrating changes which may occur.

It may be observed also that the salts of this acid contain no water, the is the acid is represented as containing only acid (or base) water as opposed to water of crystallization. The results of Conner (4) are likewise significant in this connection. He found that not only the amount of SiO$_2$ but also the amount of water in the silicates of alumina had a marked effect upon their reaction. Those silicates with very little water or very little of both water and silica were not acid, while those containing considerable of both were highly acid. This would indicate that much acidity might be due to alumino-silicic acid or acid silicates, and that silicic acid is capable of producing a lime requirement in soils.

It is not thought, however, that silicic acid can give a high hydrogen-ion concentration to soils, but the alumino silicic acids by virtue of the insolubility of their salts may liberate stronger acids, and there can be but little doubt that they take up base and are, therefore, partly responsible for a consid-

erable portion of the lime requirement shown by many acidity methods. But it is possible as suggested by MacIntire (7) that silicic acid is really a stronger acid than is commonly supposed. This author has found that large amounts of magnesium carbonate may be converted into silicates in the soil in a very short time. But it is quite possible too that such a silicate would react again in part at least to neutralize stronger acids. Sodium or potassium silicate should yield considerable free base by hydrolysis, and would, therefore, have neutralizing power. Magnesium silicate similarly to calcium should function as a strong base relative to the solubility.

But other, though less abundant, acids may be credited with the major injurious reaction of soils. Sulfuric acid which may result from the oxidation of sulfur-containing minerals such as pyrites, or sulfur-containing proteins, or by the oxidation of free sulfur, produces decidedly harmful effects if base is not present. This is very emphatically demonstrated on ammonium-sulfate treated plots where the cumulative effect of several years has been noted. The same effects may result from the use of potassium chloride. These acids are doubtless sometimes set free through an exchange of bases, such as the substitution of potassium for aluminum in zeolites (if present), feldspars or other complex soil minerals. Parker (9) in particular has observed such substitutions to occur under experimental conditions. The hydrolysis of the aluminum salt would give a highly acid reaction. According to the work of Conner (4) this is the primary cause of acidity on certain soils, and the acidity is reduced by treating the soils with phosphate which removes the aluminum and iron from a sphere of active functioning.

Nitric, though a strong acid, is perhaps not often directly responsible for an injurious soil reaction for two reasons. Nitrates are used largely by all plants and soil organisms, and are rapidly leached from the soil if not used. But leaching removes base and in this indirect way promotes acidity. If the nitrification process were very rapid there would likely result an unusual depletion of soil bases. In this same connection it is worthy of note that no highly soluble, reactive acid can long exist in the free state even in the absence of base, because of leaching. Likewise the salts of such acids in so far as they are soluble, are rapidly leached from humid soils.

A very important acid from the standpoint of plant economy is phosphoric. This is a rather weak acid being only about 17 per cent ionized in an N/2 solution. It is not likely however, that any harmful acidity may result from the use of phosphates.

When acidity is designated as a condition which results in the taking up of lime, part of such a lime requirement is undoubtedly not the result of the presence of toxic acids. According to van Bemmelen (1), though zeolitic silicates or other hydrous silicates may take up bases by exchange, not all are absorbed with the same readiness. The order given is potassium, ammonium, sodium and calcium, in descending scale. Calcium is one of the last bases to be held and should accordingly have ample opportunity for leaching,

and it is true in practice that calcium is lost by soil leaching to a much greater extent than any other base. On this account as well as for economic reasons, lime is the chief base likely to be needed for artificial soil treatment. Potassium which is used to a larger extent by plants, being absorbed readily, is leached only slightly and most soils except sands contain an abundant supply. The extent of potassium absorption is evident from the fact that in river water there is only about one-third as much potassium as sodium, while in the ocean the ratio has been reduced to one-thirtieth as much (2). The original soil not only gives up its potassium reluctantly as compared with sodium, but the river silt removes the former base much more rapidly than it does the latter and consequently it is mainly sodium which is carried into the sea. From the acidity phase of the question the above consideration is significant in that it indicates that at least a part of the base taken up by acid soils is the result of chemical absorption by complex acid silicates, which is a valuable natural check upon the loss of soil bases.

Basic minerals (those having less than 55 per cent of silica) weather much more rapidly than acidic minerals, so-called because of higher silica content rather than because of reaction. Clays are predominantly acidic and weather very slowly. According to Clark and Steiger, on the other hand, minerals such as apophyllite, natronlite and pectolite react alkaline when moistened, but such minerals give up their alkalis rather readily. Practically all minerals are soluble and subject to decay, most of them appreciably so in water, especially in the presence of carbon dioxide. Organic acids likewise increase the rate of dissolution. Oxides of iron and aluminum are the most refractory and least likely to be removed by leaching of all minerals occurring in crystalline silicious rocks. These same minerals, however, undergo many changes of combination and are a continuous possible source of soil acidity if conditions become right, for acid functioning.

In general, it may be said that an injurious mineral acidity is not only possible but highly probable in most acid soils. Or to state the situation more logically, when there is a toxic acidity in soils, the acid ion probably has its source in a mineral acid and much of the reserve acidity is likewise of the same nature. Such acids, many of them quite insoluble and not subject to oxidation, must result in a rather stable acidity. But the concentration of hydrogen-ion which is furnished and the consequent injury to plants or soil organisms is a complicated and as yet not specifically determined problem. According to Truog, it is rather the lime requirement of the plant than that of the soil which is significant. Such would undoubtedly be true on soils of weakly active acids where bases were lacking. In the presence of stronger acids in larger amounts it is probably true that a direct injury results to plants from the acids themselves. Crops often have short stubby and stunted roots when grown in acid soils evidently because the acidity is not favorable to the spreading and elongation of the root systems. But the effect of reaction upon the microscopic flora of the soil is of just as great significance as is the

effect upon higher plants. Most bacteria do not feed upon bases so that the question comes to be in this case one of reaction rather than of food supply. Such hydrogen-ion studies as have been made show that there is a more or less definite concentration which organisms are able to endure. Soils are found to have too great an acidity for certain organisms, especially nitrifying and nitrogen-fixing organisms, and under such conditions the specific organism is present only sparsely. Its presence is doubtless due to the fact that there are local alkaline or buffered regions where the organisms may multiply and to the fact that resistant strains tend to develop under acid conditions.

Organic acids

There are two distinct kinds of material which may give rise to organic acids. These are the proteins and the carbohydrates. If the proteins are considered it is found that their acid equivalent depends upon the number of free carboxyl groups, while the net acidity and reaction of the protein depends upon the relative number of carboxyl and amino groups, the one group just about offsetting the effect of the other. Proteins are probably only very slightly ionized, and for that reason would react very slowly with soil bases. But they may be present in the form of an "inner salt" and would therefore react very little with lime, until decomposed.

Proteins are made up of amino acids and when decomposition occurs it is brought about by a hydrolytic process, water being added at the peptide likage, thereby freeing the amino acids. When the decomposition process has proceeded this far there can be little effect toward increasing the hydrogen-ion concentration of the soil. Most of the amino acids such as amino acetic, amino propionic, tyrosine, lysine, arginine, histidine, and phenylalanine have acid ionization constants of an order of magnitude varying from 2×10^{-9} to 1×10^{-14} which is much too weak to have a very harmful effect. The acid constant for carbonic acid is 3×10^{-7} and it would not be expected therefore that the much weaker amino acids would very readily displace carbonic acids from its salts, and thereby use up base. But such reactions do occur and there are calcium and other salts of proteins. But these same amino acids have basic ionization constants of practically the same order of magnitude, the values varying from about 5×10^{-9} to 3×10^{-12}. Some are slightly more acidic than basic, others the reverse, but the sum total effect would be nearly neutral.

The next step in decomposition would probably be the splitting off of ammonia from the amino acids. Taking amino acetic acid as an example, it may readily be seen that the ammonia produced will be fully equivalent to the acetic acid, and the effect upon soil reaction will still remain negative, ammonia and acetic acid having practically the same ionization values. And the common organic acids which might originate in this way are of practically the same strength. Thus acetic, valeric, butyric and propionic acids have

constants of the order of magnitude of 1.6×10^{-5}. These same fatty acids
might be formed just as well from carbohydrates and in that case no ammonia
production would accompany the process, and whatever acids were produced
could increase the actual acidity of the soils. Such acids are strong enough
to give an appreciable hydrogen-ion concentration and would really be in-
jurious in case they were stable. But it is a well known fact that many organic
acids are quite volatile or readily oxidized, and it is questionable if under any
condition favorable to crop production there would be a noticeable accumu-
lation of organic acids. Organic acids are suitable sources of energy for cer-
tain soil organisms and when aeration is provided they are probably oxidized
about as they are produced. Such data as are available indicate this to be
true.

But considering again the effect of the ammonia which is liberated along
with the fatty acids in protein decomposition, as well as other nitrogen bases
which might occur, it is very evident that nitrification must occur sooner or
later. This not only removes base, but produces strong nitric acid at the
same time and should, therefore, have a marked effect upon soil reaction.
In many soils there is several times as much nitrate produced as is used by
growing crops. The nitrates are readily leached and in this way remove
large amounts of base and, therefore, tend to cause base-poor soils. This
is another reason why tilled soils may become acid rapidly. Virgin soils
nitrify much more slowly as a rule, and there is, therefore, more of the buffer-
ing effects of accumulated proteins as well as less leaching of bases in the form
of nitrates.

The general indication is, therefore, that organic matter is not likely to
produce a harmful soil acidity. During the wet season when the soil is satu-
rated and oxidation cannot proceed readily, there may be an accumulation of
organic acids. But when dry conditions prevail these acids rapidly oxidize
and disappear. The general effect of organic material would seem to be to
keep down acidity rather than to increase it. Proteins are good buffers,
which means that they are capable of combining with either acid or base
without changing in reaction. If the reaction is with stronger acids there
is a reduction of the acidity in terms of hydrogen-ion. If they combine with
base, it is only prevented from leaching, since many protein salts as well as
organic acid salts are quite insoluble and non-leachable. But decomposition
will finally liberate the base and it may then neutralize a strong acid. Thus
calcium citrate or oxalate should be just as effective as lime in neutralizing
sulfuric acid, since either of these organic acids would be expected to disap-
pear from the soil quite rapidly when liberated.

The question may arise why peat and muck soils should become very acid.
In reply it may be stated that conditions are very different here from those
found in ordinary soils. The content of organic matter is very high, aeration
is usually not good, since such soils occur only in low and wet places. And
mineral bases are usually not present. Where bases are present acidity does

not occur. Such soils, therefore, become acid because conditions are extremely favorable to the large production of organic acids, with little opportunity for their removal. In general, such soils would be expected to take up large amounts of lime, even though the hydrogen-ion concentration might not be high.

Colloids and soil reaction

As stated above, soil acidity has been attributed to adsorption of bases by colloids, though this explanation is usually thought to be entirely inadequate. A question naturally arises as to why and how colloids may contribute to acidity. The answer may possibly be found in the size of colloid particles.

It may be stated that practically any material may form a colloid if obtained in the proper state of divisions. Colloidal particles are larger than ordinary molecules and yet not large enough to settle out. The average molecule is estimated at 2×10^{-8} cm. while the range of colloids is usually placed at from 0.1μ to 1.0μ μ, which is 1×10^{-5} to 1×10^{-6} cm. There may be, therefore, several thousand molecules in one colloidal particle. On the other hand, single molecules, such as the hemoglobin molecule which is 2.3 to 2.5×10^{-6} cm. in diameter, may be large enough to form a colloid.

The effect of a colloid, therefore, is greatly to increase the surface of any liquid in which it may be suspended. And since adsorption is a surface phenomenon, the relation to colloids becomes evident. This relationship may be further demonstrated as follows (5): Suppose that 1 cc. of a substance is reduced to cubes 0.1μ on each edge and suspended in water. The volume of one cube becomes $0.1\mu^3$ or 1×10^{-15} cc. The number of particles, therefore, is 1×10^{-15}. The surface of one particle is $6 \times (0.1\mu)^2$ or 6×10^{-10} sq. cm. and the total surface is 6×10^5 sq. cm. The surface of the original cube was only 6 sq. cm. Therefore, the surface of the water suspension of the divided particles is increased 100,000 times. The surface tension of water expressed in dynes per square centimeter has likewise been increased 100,000 times. Then since those substances which reduce the surface energy tend to accumulate in the surface, a much larger quantity of substances low in surface energy can be adsorbed. According to the adsorption theory of soil acidity, bases reduce the surface energy and therefore, are adsorbed and removed as though they had been taken up by acids.

If this were the correct theory for soil acidity, such a phenomena could be produced by any sort of finely divided material, and it should be possible to create an acidity, increasing or decreasing it at will by varying the quantity and size of colloidal particles in suspension. No data of this kind are available.

And again it may be observed that such substances as fatty acids and other organic materials have a very low surface energy, acetic acid having an energy of 23 dynes compared to 73 dynes for pure water at the temperature of 20°C. If organic acids did occur in soils they would, therefore, be adsorbed to a much greater extent than mineral bases. There seems to be

no surface tension data for the alkalis, but the surface tension of mineral aci
such as sulfuric and hydrochloric and their salts, is greater than that of p
water, varying from about 74 to 80 dynes per square centimeter. Eviden
therefore, seems to be rather against the theory that the adsorption of ba
is responsible for what is commonly called soil acidity, though it is entir
possible that true acids or bases present in the soil from whatever sou
may be adsorbed and held in a very similar way as hygroscopic or soil fi
water.

There is no question but that colloids of both mineral and organic nat
are present in abundance in soils, and that they play a part in soil acid
through chemical action. Colloids are natural phenomena of rock formati
The original molten magma, which was a mineral solution, contained
various ions which reacted with each other to form the complex silicates
aluminum, such as feldspars and feldspathoids which by subsequent alte
tions, produced soils. In the cooling of the magma various minerals, acco
ing as their fusing temperature is low or high, separated out at certain interva
the feldspars and silicic anhydride being probably among the last to separa
If the cooling was very rapid a glass or highly viscous colloid separated.
the cooling was slow enough, crystallization occurred. As a matter of fa
the formation of a colloid may be thought of as a process of crystallization
which most of the crystals formed are infinitely small.

The most important mineral colloids of soils are the clay and zeolites a
similar hydrated silicates or secondary products of rock formation, and t
less important extent hydroxides of iron and alumina and sometimes sili
acid. It is characteristic of colloids to be rather highly hydrated, the wa
being held loosely, chemically combined and ionizing sometimes as an a
and again as a base. The amphoteric nature of colloids is due to their abil
to undergo the two kinds of ionization, the particular kind undoubtedly
pending on several factors. The presence of strong acids would, of cou
favor the basic ionization.

There is little question but that colloids exhibit a capacity for taking
bases. But it is seldom that a colloid may give a toxic hydrogen-ion c
centration, and whether there is a physical adsorption by colloids is an o
question. According to Langmuir (6) adsorption is entirely chemical,
adsorbed material being held by chemical forces exerted upon active grou

such as $^-$OH, $=$ CO, $^-$C$\underset{\text{O}_-,}{\overset{\text{O}}{\diagup}}$ tc. present in the adsorbed molecules. T

chemical force is active in the surface layer of the adsorbing surface. 1
adsorption theory of Harris depending upon the Helmholtz double la
hypothesis is likewise really chemical, since there is an exchange of ions a
chemical forces operating to hold the adsorbed ions. It may be conside
at least true that a colloid will take up base in so far as it ionizes as an ac
but such a reaction is only an ordinary chemical phenomenon.

Practically all organic materials of the soil are colloids, and mostly of the emulsoid type. An important distinction between suspensoid and emulsoid colloids is found in the amount of water of hydration. Thus albumin may hold many times its weight of water. Mineral colloids, on the other hand, rarely hold more than 10 to 20 per cent as bound water. This affinity for water is responsible for a number of colloidal properties. Thus the swelling of soils is due to the taking up of water by soil colloids, and the shrinking and cracking is due to reverse conditions, loss of water. The large amount of water of emulsoid colloids also causes the colloid to have nearly the same index of refraction of light as the pure water in which it is dissolved, which means that the solvent and solute are nearly homogeneous. The large amount of water present causes such colloids to function as a solvent for mineral nutrients.

The reaction of most colloidal material of the soil is nearly neutral or only slightly acidic. Partly for this reason and partly because of food supply organisms are more numerous and active in the presence of organic matter. It is possible, however, to have a highly organic soil which is not active bacteriologically. This condition occurs in peats and forest soils and is usually attributed to acidity. Acid-resistant fungi such as molds are characteristic of such soils.

It is probable that in mineral soils, organic materials are present as protective colloids about the fine mineral particles of the soil mass. In this organic layer or film, organisms are active, and it is likewise here that soil reaction functions. It is generally found that burning a soil destroys its acidity and makes it an especially suitable medium for bacterial action. This is likewise easily explained when the nature of colloidal material is understood. Mineral silicates and also organic colloids are dehydrated by burning. Since the colloid owes its acid properties to its water of hydration, it is quite natural that acidity should be destroyed by heat. Other stronger acids such as nitric would be either volatilized or converted into harmless salts by bases in the ash of the organic constituents. Then, too, since colloids have the property of swelling and because of their fineness of division, of filling all interspaces of soil particles, aeration of the soil is much increased by burning. Ignited soil then becomes an excellent medium for aerobic organisms and is often used for their study in the laboratory.

CONCLUSION

In the natural processes of soil formation such as the kaolinitization of feldspars, bases are removed, tending to leave acid alumino-silicates, or silicic anhydride capable of rehydration to acid silica, with the result that mineral soils may have a comparatively large reserve of slowly reactive acids. Such acids are capable of a more or less indefinite but continuous decomposition of carbonate.

Toxic acid reactions, however, are more probably due to the presence of more soluble and highly ionized acids, such as sulfuric or hydrochloric, nitric, etc. usually introduced by some sort of fertilizing treatments. Such acids exert toxic action because of previous removal of base primarily by leaching. It is only seldom and in highly organic, poorly drained soils that organic acids accumulate to a detrimental extent. This may be observed in peat soils but not often in soils of mineral types. In fact, organic matter quite likely depresses the more active acidity by buffering and by supplying base in the form of ammonia or its derivatives.

The phenomena of absorption of base, commonly classed as acidity, is a very desirable action resulting as it does in the fixation of elements otherwise lost in the drainage. Even though fixation occurs automatically, the hydration and hydrolytic changes which are constantly occurring permit base weathering with the loss of essential elements and an increased tendency to give an active soil acidity.

The method of formation of soils and the susceptibility to erosion and leaching are probably important factors in the production of acid soils. Thus soils formed by the slow weathering and accumulation of rock debris, *in situ*, would allow ample opportunity for base leaching even though large amounts were originally present. On the other hand, glacial soils being deposited more rapidly if they contained basic material originally, would probably not become acid so quickly, that is, they were not extensively base-leached during the process of formation.

In a somewhat similar way tillage must increase the tendency to develop acidity, for crops remove base, and tillage increases bacterial activity, thus producing more carbon dioxide to facilitate leaching. Tillage also exhausts organic matter, thus removing part of the soil capacity for holding base and destroying its buffering propensities. Active nitrification is especially exhaustive of base. A very effective safeguard against an injurious acidity should be to maintain an adequate supply of organic matter in the soil.

SUMMARY

1. The modified Tacke method is capable of adaptation to determinations of the relative activities of different fractions of soil acidity.

2. The more reactive acids, those capable of giving a toxic concentration of hydrogen-ion, react rather quickly. This has been proved by the application of lime to acid soil in successive increments and applying a qualitative test to determine at what stage a neutral soil reaction may be obtained.

3. Soils may contain a large reserve of acidity, which might be described as potential rather than active, but which is, nevertheless, capable of slowly decomposing carbonates.

4. Rather highly buffered solutions react rapidly with calcium carbonate, even in the presence of soil, indicating that even the more active fractions of the soil acids may be considerably buffered.

5. Protein materials and amino acids so far as tested, previous to decomposition in the soil, do not react readily with carbonates.

6. Knowledge of acid soils is considered more adequate when something is known of the activity of the acids, as well as of the total potential acidity commonly determined, more or less inaccurately. It is not the capacity of a soil to decompose lime, but rather the intensity of decomposition which is most highly significant.

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THE VOLUMETRIC DETERMINATION OF SULFATES BY OXIDATION OF BENZIDINE SULFATE WITH KMnO₄

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The main principles of the method here given are similar to those proposed by Raiziss and Dubin (2), but there is much modification of details in order to increase accuracy. Its essentials are: first, the isolation of SO₄ as the insoluble benzidine salt, second, the measurement of the amount of combined benzidine by titration with standard permanganate. This is carried out at boiling temperature with excess of KMnO₄. The excess is removed by standard oxalic acid, and the final titration completed with KMnO₄. In this way inaccuracies due to variations in temperature, time, concentration, and incompleteness of oxidation are largely eliminated. Raiziss and Dubin gave some attention to the effect of these variables, but did not define precisely the conditions required for high accuracy.

For the experimental work here reported a solution of benzidine hydrochloride containing 8 gm. in 1 liter was used. Two solutions of K₂SO₄ were used as source of SO₄, one containing 1 mgm., the other 0.1 mgm. SO₄ in 1 cc. Titrations were made with N/20 KMnO₄ and against this N/20 oxalic acid was used. The exact strength of KMnO₄ was found by Soerensen's sodium oxalate.

In order to determine the effect of varying conditions, 10-cc. portions of the benzidine solution were titrated with N/20 KMnO₄ under varying conditions.

It was found that temperature should be kept constant in order to secure concordant results, while variation in free H₂SO₄ is not important. Variation in volume has somewhat the same effect as variation in temperature due to lowering of temperature by the cold KMnO₄ when the volume of the solution to which it was added was small. To eliminate as much as possible the effect of variation in temperature and volume it was decided to start the titration with a constant volume of 100 cc., containing 5 cc. H₂SO₄ and heat in a boiling water bath, which would require no special apparatus or attention to maintain a constant temperature.

Variation in the amount of benzidine in the solution to be titrated was found to vary the factor for converting cubic centimeters of KMnO₄ used to SO₄, if the method of titration given by Raiziss and Dubin were used. This method does not carry the reaction to completion, especially if much benzi-

dine is present. Christie and Martin (1) applied this method to the determination of SO₄ in soil extracts, using the factor 0.15 to convert cubic centimeters of N/20 KMnO₄ to mgm. SO₄. With this factor, small amounts of SO₄ are underestimated, while large amounts are greatly overestimated. Applying this method on known amounts of sulfate, the factor for N/20 KMnO₄ was found to be as follows: for 1 mgm. SO₄ 0.179; for 2 mgm. SO₄, 0.152; for 5 mgm. SO₄, 0.133. With the last it was impossible to carry the reaction to completion in any reasonable time. A slight excess of KMnO₄ soon disappears, and this may be repeated indefinitely for some time.

When the new method—excess KMnO₄, digesting hot, adding excess oxalic acid, and completing the titration with KMnO₄—was used, the factors found were: for 1 mgm. SO₄, 0.124; for 2 mgm. SO₄, 0.122; for 5 mgm. SO₄, 0.122. Even with this method the reaction is not quite complete in the presence of 5 mgm. SO₄. With amounts less than 2 mgm. SO₄ the end point is quite sharp. In fact it is the same as in the titration of pure oxalic acid. The titration is completed in a few seconds, and an excess of 1 drop KMnO₄ colors the solution pink for some time.

Imperfect washing of the precipitate of benzidine sulfate may cause many errors in the determination of SO₄ due to the uncombined benzidine remaining in the precipitate. Considerable time and effort were spent in the endeavor to improve the washing process. The precipitate is somewhat soluble in water, more so as the water is warmer, and considerably soluble in alcohol. Addition of small amounts of acids, hydrochloric, nitric, phosphoric or acetic, to the wash water, seemed to increase the solubility of the precipitate. It is decomposed by dilute alkalies. A saturated water solution of benzidine sulfate does not dissolve the precipitate, but instead increases its amount, thus causing plus error. It was finally decided that water was the only feasible washing agent and that the amount used must be very small and as cold as possible in order to avoid solution of the precipitate.

All the details of the procedure are given in the following description of the method. At best it must be admitted that it is somewhat empirical and has serious limitations, yet it is rapid and sufficiently accurate for most work on soil extracts, biological fluids or other similar solutions containing small amounts of sulfates.

PREPARATION OF THE SULFATE SOLUTION

The solution should be free from organic matter, which hinders complete precipitation; iron, which causes various irregularities; phosphate ion, which is precipitated by benzidine; nitrates, which increase the amount of precipitate and other oxidizing agents or heavy metals. It may be prepared by evaporating the soil extract or similar solution to dryness in a porcelain casserole and igniting. One or two cubic centimeters of nitric acid added before evaporating will greatly assist in burning off organic matter. If there is much,

the precipitated manganese has redissolved and the solution is colorless, complete the titration with the $KMnO_4$. One drop in excess should give the solution a pink color permanent for some time. However, if more than 3 mgm. of SO_4 were taken, oxidation will be incomplete under these conditions and the pink color will soon fade. The total number of cubic centimeters of $KMnO_4$ used, less 10, multiplied by 0.12 gives the number of milligrams of SO_4 in the solution taken. On account of the inadequate washing and other variables a blank determination will require 0.3 to 0.7 cc. $N/20$ $KMnO_4$. Using the factor 0.12, quantities of SO_4 less than 1 mgm. will be likely to be slightly over-estimated, while quantities over 3 mgm. are likely to be under-estimated. In order to determine the exact factor to be used, it is only necessary to run through the process with a known amount of sulfate of about the same magnitude as that in the substance analyzed.

For the sake of completeness, the following notes are added. Theoretically 1 SO_4 required about 2.72 benzidine hydrochloride to precipitate it. The solution used contains 40 mgm. in 5 cc., which should precipitate 14 mgm. SO_4, much more than can be well handled. A considerable excess of the reagent is desirable, but the amount used is ample. However, it was found by direct experiment that a much larger excess only slightly influenced the result.

A saturated water solution of benzidine sulfate for washing the precipitate is not successful, probably on account of the large variation of solubility with variation of temperature.

Free acid should be kept near the limits suggested, as the precipitate is notably soluble in dilute acids.

Iron in the solution makes it difficult to adjust the acidity properly and develops various colors leading to erratic results.

Phosphate is precipitated by benzidine less readily than sulfate, especially in the presence of free acid. But it is so difficult to adjust the acidity in order to precipitate SO_4 while holding PO_4 in solution, that it is best to remove PO_4 if the amount present is more than 0.3 mgm. or sufficient to make a visible precipitate when made alkaline.

The degree of accuracy obtainable is indicated by the following figures:

SO_4 TAKEN	SO_4 FOUND BY USE OF FACTOR 0.12
mgm.	*mgm.*
0.5	0.492
0.5	0.528
1.0	0.962
1.0	0.996
2.0	2.040
2.0	2.016
5.0	4.860
5.0	4.980

SUMMARY

1. The method proposed by Raiziss and Dubin is faulty in that the conditions are not sharply defined, so that the ratio of SO_4 to $KMnO_4$ is not constant, but varies with the amount of each, and with other varying factors.

2. Temperature and volume are the most important factors which should be kept constant.

3. The method of Raiziss and Dubin never carried the oxidation of the benzidine to completion, and the less so, as more benzidine was present.

4. The new method, here proposed, secures complete oxidation, a sharp end point, and a constant ratio of SO_4 to $KMnO_4$, by proper control of conditions.

5. A perfectly satisfactory method of washing the precipitate of benzidine sulfate has not been found.

6. The method is described in detail. It is rapid and accurate if properly executed.

7. The solution for precipitation of benzidine sulfates should be nearly free of organic matter, which may be removed by evaporation with aqua regia to dryness in a flask, or by evaporation to dryness and ignition in a porcelain dish. Iron, nitrate, phosphate, and heavy metals should be absent.

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VEGETATIVE GROWTH IN SOILS CONTAINING CRUDE PETROLEUM

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The writer had occasion during the past year to make some investigations concerning the effect upon vegetative growth of crude petroleum oil mixed with soil. The occasion for the oil coming in contact with the soil was due to breaks in cross-country pipe lines which permitted some oil to escape to the surface of the ground and spread, or be carried by high water, and later backed over the lower lands. Investigations of the soil and crops said to be damaged by oil were made with a view to using the data in a land damage suit. There is plenty of evidence of the killing effect of crude oil when in contact with growing plants, but the writer was unable to get data on the damaging effect of varying amounts of crude oil which had been incorporated in the soil from any cause, and the following year this soil was prepared for growing a crop. It was claimed by one witness in the course of the damage suit that the soil was "killed" by the oil, whatever that may mean. It might be supposed the killing referred to meant destroying bacterial life in the soil and making it useless in further crop production.

VEGETATION EXPERIMENTS

Since no data bearing directly on this problem were available, it was thought to be worth while to carry on some vegetation tests to determine how much oil may be incorporated with the soil and not render it useless for crop production or kill all the bacterial life in the soil.

Plan of experiment

Unglazed earthen pots, holding about one gallon, were filled (8.8 pounds) with air-dry, sandy peat soil similar to that under litigation, and tests were run in duplicate. The oil was thoroughly mixed with the soil, only 5 drops being added at a time, and then well stirred. The soil was then wet with water containing soybean bacteria. After standing for 2 days soybean seeds were planted. Table 1 gives some details of their growth and maturity.

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TABLE 1

Growth of soybeans in soil containing varying amounts of crude oil

POT NUMBER	OIL PER GALLON OF SOIL	TOTAL NUMBER OF PODS PRODUCED	TOTAL WEIGHT OF PODS	AVERAGE WEIGHT PER POD	NUMBER OF STALKS	NODULES PER STALK	DRY WEIGHT OF 3 STALKS	PER CENT OF OIL ADDED TO SOIL	OIL ADDED PER ACRE (2,000,000 LBS.)
	cc.		gm.	gm.			gm.		lbs.
1	Blank	40	14.0	0.35	3	25	25	0	0
2	5	39	16.0	0.41	3	20	26	0.12	2,200
3	10	32	12.1	0.38	3	30	24	0.25	4,400
4	20	33	12.5	0.38	3	28	24	0.50	9,000
5	30	84	38.0	0.45	3	108	61	0.75	13,400
6	40	35	14.0	0.40	3	48	26	1.00	18,000
7	50	33	12.5	0.38	3	48	24	1.25	22,400
8	60	32	13.0	0.41	3	96	23	1.50	27,000
9	70	25	8.0	0.32	3	29	14	1.75	31,400
10	80	18	4.5	0.25	3	23	15	2.00	36,000
11	90	31	10.0	0.32	3	32	26	2.25	40,400
12	100	15	5.0	0.33	3	12	9	2.50	45,000
13	120	15	4.3	0.29	3	30	9	3.00	54,000
14	160	3	0.4	0.13	3	7	8	4.00	72,000

DISCUSSION

It will be noted from the table that the growth of soybeans was apparently improved through the addition of small amounts of oil (30 cc. per gallon, 0.75 per cent) and that rather large amounts may be mixed with the soil (160 cc. per gallon, 4.0 per cent) before the soybean plant succumbs to the oil treatment. The damage seems to be due in part to the plant's inability to secure water rapidly enough to meet its needs.

It would appear from the table that a small amount of oil is even desirable in nodule development in the soybean plant as compared with the check pots, and where the amount of oil was increased to the extent of damaging the plant, there was still some nodule formation.

ACID SOILS AND THE TOXICITY OF MANGANESE

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A study of the peculiar behavior of certain plots on the Alabama Agricultural Experiment Station Farm led the writer to conclude that soluble manganese produced by the action of nitrogenous fertilizers was the cause of the toxicity of these soils, and of their water extracts. The results on which this conclusion was based were published in Bulletin 201 of the Alabama Agriculture Experiment Station.

Further studies on these plot soils and on others from various places in Alabama indicate that the earlier conclusion is untenable, since the addition of manganese carbonate to such toxic extracts has produced conditions favorable to the growth of seedling pea roots in every instance. Acid soils when incubated with dried blood and manganese carbonate gave extracts which contained more soluble manganese than when incubated with dried blood alone, but were not nearly as toxic. The injury resulting from large amounts of manganese, under these conditions, is more apparent on the leaves than on the roots of seedlings; the leaves being bleached and abnormal in shape, whereas the roots make nearly a normal growth. Moderate amounts of soluble manganese in extracts of soils incubated with dried blood and manganese carbonate caused no apparent injury to either roots or tops of pea seedlings.

The accumulated evidence at hand indicates that the toxicity observed in certain soils and soil extracts, after incubation with a nitrogenous fertilizer, is possibly due to uncombined acid, or more probably to readily hydrolyzable salts. Hydrogen-ion determinations made on such toxic extracts showed that in all cases the H-ion concentration was relatively high. Further details are reserved for a later publication.

CULTURAL STUDIES OF SPECIES OF ACTINOMYCES ·

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CONTENTS

INTRODUCTION

The actinomycetes form a large and important group of microörgai both in numbers and activities. They have been isolated from wound water and foodstuffs, but chiefly from the soil. Their functions are as va as those of any other large group of living forms, so that we cannot po any particular activity as their probable rôle in nature. As any other group of forms of life, they are extremely variable: both their morpholog physiology vary with the environmental conditions, and any classifi which is based upon the cultural or morphological studies on one medi artificial and will not accomplish any far-reaching results in establishi identity of the species.

Many actinomycetes, chiefly the pathogenic forms (animal), were s1 on complex organic media, upon which they produced an abundan1 uncharacteristic growth. The basis for study was necessarily the physiol activities of the organisms, but these, as will be pointed out soon, canr used as a basis for classification, when only a few strains are available. characteristic morphology of the actinomycetes is brought out only on s

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* Colored plates to illustrate this article were prepared by the author, but on a of lack of funds it was necessary to omit them. It is hoped they will be publisl some later date.

synthetic media, particularly when the nitrogen and carbon sources do not allow any abundant growth of the organisms, so that they do not form much of a substratum growth (which is uncharacteristic in most cases) but are found to produce an aerial mycelium, characteristic for the different organisms, and thus allow a detailed study of their morphology.

Slight differences in physiology or cultural characters have often served for the differentiation of species. When a large number of actinomycetes are studied, as in the case of the present investigation, where over 300 isolations were made, and numerous cultures obtained from other sources, these studies lasting for over four years, it will be found that differences observed are often quantitative rather than qualitative in nature. Several strains belonging to one group may show at one time differences on one medium but not on others, and, on repeated cultivation, this difference may disappear and another difference may arise. These minor changes depend on the slight variation in the composition of the medium, amount of inoculum added, form of inoculum (spore material, surface or subsurface growth), age of culture, length of time since it was isolated from its natural habitat, etc.

All the cultures should be divided into groups, the representatives of which have common morphological, physiological and cultural characters. These species groups may show slight variations within the groups, when several representatives are compared, but all of them possess in common the main distinguishing characters of the species, and are distinctly different from any other species group. Quite often cultures are obtained which form transition steps between two different species. In that case they are either classed with the species to which they approach nearest, or left as transition forms, or, if distinct enough from the two nearest species, placed into species by themselves. Another difficulty in establishing species is encountered in attempting to fix the amount of variation necessary to constitute a new species. The difference of various strains in the same groups is chiefly that of degree only.

Certain cultural characters may either be lost or gained on continued cultivation (such as pigment production on gelatin or rapidity of liquefaction, etc.) and it is, therefore, advisable to describe species of actinomycetes soon after their isolation from their natural habitat and after prolonged cultivation upon artificial culture media.

HISTORICAL

No attempt will be made here to review the numerous investigations published on this group of organisms. Attention will be called here only to the more important works which contributed largely to the advance of our knowledge on this group of organisms, where also a complete bibliography of the previous investigations can be found.

Cohn (8) first described (1875) an Actinomyces under the name of *Streptothrix Foesteri*. But this name (*Streptothrix*) is untenable, since it was pre-

empted by Corda for a genus of true fungi. The next species *Actinomyces bovis* was described by Harz (21) in 1877, and the name used by this investigator has good claims to priority and will therefore be used throughout this work; Harz placed *A. bovis* among the Hyphomycetes. Trevisan (36) applied, in 1889, the term *Nocardia* to a saprophytic form. This term could be used, if the organisms belonging to this group could be divided into 2 subgroups, parasitic and saprophytic, as attempted by the committee of the American Bacteriological Society on Generic Classification, but this subdivision cannot hold, since no distinctive differences could be found both culturally and morphologically, between these two groups and the mere fact that one species was isolated from the soil and another from a wound cannot justify their placing in two distinct genera.

Domec (12) gave a detailed description of the morphology of *A. bovis* (Harz) together with an accurate account of spore formation; he demonstrated that these are really the spores of a mold fungus not of a fission fungus.

Nadson (29) isolated several species of Actinomyces from the curative mud of the Slavian mineral waters in Russia and suggested that the genus should be placed in a group of fungi by itself.

Gedoelst (18) placed this group among the Fungi Imperfecti, in the genus *Discomyces* Rivolta (1889), with *Streptothrix* Cohn (1875), *Actinomyces* Harz (1877), *Nocardia* Trevisan (1889), *Oospora* Savagean and Rádais (1892) and *Discomyces* Blanchard (1900) as synonyms. Petruschky (33) classified the actinomycetes with the *Trichomycetes* of the hyphomycetes, recognizing 4 groups: *Actinomyces*, *Streptothrix*, *Cladothrix* and *Leptothrix*. A thorough discussion and criticism of these early attempts of classification can be found in the work of Lehmann and Neumann (24), Lachner-Sandoval (23) and those mentioned in the following pages. Musgrave, Clegg and Polk (28) in 1908 gave a detailed study of the pathogenic forms known and almost a complete bibliography.

Claypole (7) was among the first to call attention to the great variability in morphology and cultural characters of this group of organisms, which is the cause of confusion and diverse opinions and practices. These organisms change their appearance on different culture media. She suggested that this group, with their variable morphology and close relationships, should be looked upon as representing the ancestral type of both the higher fungi and the true bacteria.

The production of a pigment by the actinomycetes has early attracted the attention of the investigators as a basis for classification. Gasperini (17) thought to have definitely established the identity of *A. chromogenus*, which is supposed to produce a brown pigment in gelatin or peptone agar, which rapidly diffuses through the medium. Krainsky (22) and later Waksman and Curtis (44) and Conn (10) have shown that the pigment production is an insufficient basis for the characterization of the species. A very large number of strains of this group of organisms can produce a brown (of different shades)

pigment on gelatin, potato and peptone agar; this property, as was pointed out above, is not a constant characteristic for all types. Sanfelice (35), recognizing the great variability of these organisms, suggested a division into groups, but he used as a basis for classification the color production upon complex organic media; had he used synthetic media, standard in composition, this division might have been of some value, but the pigment production upon complex organic media is a very indefinite basis for any attempt to subdivide the organisms.

Krainsky (22) recognized this difficulty and was the first to suggest the use of simple synthetic media, standard in composition, for the study of this group. He described 18 species of saprophytic actinomycetes which are undoubtedly the most complete descriptions that appeared in all the literature; but even his work has not been sufficient enough that one would be able to identify with certainty any species that he has described.

The writer with Curtis (44) published a preliminary report on the actinomycetes in the soil, of which the following work, after a careful and continuous study for 4 years, is a result. They attempted to classify the actinomycetes on the basis of liquefaction and pigment production in gelatin and spiral formation in aerial mycelium. This method was justly criticised by Conn (10), who however did not suggest anything more definite; if anything, his cultural studies, adding some information, cannot go toward classification even as far as the division into types producing macro and micro colonies, as suggested by Krainsky (22), or spiral formation and liquefaction and pigment production in gelatin, used by Waksman and Curtis (44). The latter stated that some species that they isolated from the soil were the same as those isolated by Krainsky (22), but since the comparison was based only on description of the latter's cultures, certain differences were found on further study; but several species, such as *A. viridochromogenus*, were found to correspond very closely with the descriptions of Krainsky on all media. A few cultures were either lost on transfer since the first description, or were found to fall into one group with others, with which they were properly placed.

Conn (10) mentioned three main types of soil actinomycetes and described one new species. Waksman and Curtis (45) demonstrated the wide occurrence of actinomycetes in soils under different conditions of climate, topography and cultivation. Certain species (or groups) were found to be very abundant in the soil and were isolated from soils located in different parts of the world, while others were isolated only once or twice. The writer (41, 43) recently published a series of investigations on the metabolism of the actinomycetes, and some of the data published there will be repeated here for the sake of completeness of the work.

Drechsler (13) has made the most complete morphological study that we have so far of the genus Actinomyces, and concluded that it should be classed with the Hyphomycetes, as a Mucedineous group with tendencies toward an erect Isarioid habit. If the bacteriologists that studied this group can be criticised

for paying little attention to the true morphology of the organisms, the
nists should be criticised, when studying the morphology of a large gro
microörganisms like the actinomycetes, in entirely neglecting the effect
vironmental conditions, particularly the composition of media.

It has been repeatedly pointed out (25, 43) that the composition of th
ture medium affects the character of the colonies of bacteria and even
to such a degree as to make it necessary to have media of a standard che
composition and to have an exact description of these media. Partic
such a variable group as the actinomycetes, which give growth distinctly
ent in many respects, with a mere change of the source of nitrogen or ca
should be cultivated on media of exact chemical composition, especially
exact morphological characters are studied. The most excellent morph
cal studies of Drechsler have this one disadvantage—they have been c
out on media of variable composition (peptone glucose agar and potato
at an unknown temperature and for an unknown length of time. Any
these factors will affect the biochemical and cultural characters, as w
the morphological.

NOMENCLATURE

The generic name

The systematic position of this whole group of microörganisms has bee
cussed before. All that we know about their structure and development
make us recognize them as true fungi—*Eumycetes* Brefeld. They prod
very fine, unicellular mycelium, well developed and abundantly brar
They reproduce by means of aerial spores, or so-called conidia, which
nothing in common with bacterial forms of reproduction, but resemble g
those of many fungi. They are easily separated from the bacteria by
morphology as well as cultural characters; they are differentiated b
true branching, character of growth and type of conidia from the ba
characterized by the formation of false branching, such as *Cladothrix* and
producing involution forms (*B. tuberculosis*).

They cannot be classified with the fungi *Oospora*, *Streptothrix*, or
myces for the obvious reason that those forms are multicellular. The tern
nomyces Harz is therefore the most valid, particularly since it charact
the type of growth. The suggestion of Lechner-Sandoval (23), Leh
and Neumann (24) and Drechsler (13) to classify the genus in an unqu
manner with the Hyphomycetes will meet certain objections. The loo
of the group Hyphomycetes, where botanists have piled together forms
could not be placed elsewhere, and the fact that these have a septated
lium, will make the place for the actinomycetes improper, and it will no
much to advance the question of the proper classification. The auth
only agree with Nadson (29), who stated yet in 1900 that the actinom
form a special group of fungi, to be classified separately, until new fact
·cerning the history of their development are found.

Nomenclature of the species

As a basis for the nomenclature of the species, the organisms described by the writer and Curtis (44) and pathogens obtained from known sources were taken. The well described cultures of Krainsky (22) could not be obtained and use had to be made only of his descriptions; the identifications in that respect may be questionable, particularly in certain cases. *A. pheochromogenus* was isolated by the writer from the soil, but was first described by Conn (10). *A. poolensis* was obtained from Dr. Taubehaus (37) and also isolated from the soil. A few new species are described so as to make the study of the group complete, although, as stated above, care was taken to exclude as many of the types which approached the described species as possible. Attention will be called to this fact in the process of the work. It cannot be contended that all these species are new to science, since it is possible that certain of them have already been described before. But the fact that all the old cultures are unavailable for examination, while this publication applies to definite material, with less probability for confusing the different species when studied in one laboratory, will justify this course.

This paper lays no claim to completeness as a monograph of the genus Actinomyces. As many authentic cultures were secured as possible and careful study was made of all the published descriptions. This paper represents cultural and biochemical studies continued for over four years at several institutions and includes only those species for which the data obtained abundantly justify the characterization. Most forms were isolated by the author from the soil, a few others particularly the pathogenic forms, were obtained from other sources, as will be pointed out in the description. In this work the author was assisted during 1915–1916 by Mr. R. E. Curtis at the New Jersey Agricultural Experiment Station, who deserves a great deal of credit for the cultivation of the organisms and some biochemical studies; the work was then continued by the author during 1916, 1917 and 1918 in the Department of Biochemistry, University of California and at the Cutter Biological Laboratories, Berkeley, Cal. The work was completed during the college year 1918–1919 at the New Jersey Station, where the writer was assisted by Mr. Jacob Joffe in carrying on and checking up some cultural and biochemical studies and by Mr. Willem Rudolfs in making microscopic studies of the morphological characters of the organisms. The author takes here the opportunity to express his sincere thanks to Mr. Curtis, Mr. Joffe and Mr. Rudolfs, to the institutions where the work was carried on, and to Dr. Charles Thom and Dr. H. J. Conn for reading the manuscript.

OCCURRENCE OF ACTINOMYCETES IN NATURE

Actinomycetes have been isolated from numerous sources: as animal and plant pathogens, air, water, milk, salt water lakes and soil. One reason for their wide occurrence is the ease with which they can adapt themselves to a

new environment. Certain forms will grow in salt water or ordinary tap water without the addition of any nutrients; this is the reason why certain investigators may be led to suspect that they can fix atmospheric nitrogen. Also, the spores are very light and can easily be carried in the air for a long time. The ease of adaptability can be seen from the fact that when 3 animal pathogens, *A. bovis*, *A. madurae* and *A. hominis* were inoculated upon sterile soil to which some nitrogenous organic material (½ per cent of dried blood) had been added, they made a good growth upon it and were subsequently reisolated from the soil. The ease with which they grow on organic media is due to their ability to decompose organic substances readily. For a complete study of the occurrence of the actinomycetes in nature, reference can be here made to the work of Musgrave and associates (28), where the pathogenic forms are studied, and to the studies of Krainsky (22), Waksman and Curtis (45) and Conn (10) for the occurrence of saprophytic forms.

MORPHOLOGICAL STUDIES

Very little morphological work (Neukirch (30)) has been done on this group of organisms before that of Drechsler (13); this was due to the minuteness of the typical characters of these organisms, but primarily to the fact that most media used allowed a rather uniform development. Nadson (29) pointed out as early as 1900 (work published in 1903 and apparently overlooked by subsequent investigators) some of the typical morphological characters of these organisms: hyphae are thin, colorless, cylindrical, 0.5 μ to 0.7 μ in diameter, the growing portions being only about 0.3 μ, straight or forming spirals; hyphae branch abundantly, the branching being true, similar to that of fungi and distinctly different from the false bacterial branching (such as *Cladothrix dichotoma*); the mycelium is not septated. The contents of the young hyphae are found, under the microscope, to be pale; homogeneous granules appear in older cultures and in places the plasma breaks up into elongated portions, separated by more colorless intervals. The aerial hyphae break up into branching lines of spores, usually called aerial conidia, but more properly termed, by type of formation, oidia; these are short elliptical 0.75 x 1.25 μ. Numerous involution forms are found in older cultures, both in substratum and aerial mycelium including the conidia. The hyphae are thin regularly cylindrical, turning wide in many places, forming club-like balloons; these swollen portions of the hyphae are often transformed into a series of spherical or elliptical ampules, united by thin channels, the end of the hyphae may develop into a club-like form; this, as well as those following, may be separated from the hyphae into a free spherical body; in other cases the hyphae, becoming thicker (1.25 μ–1.50 μ), develop into screw-like forms or spirals, which may freely separate from the hyphae from which they originated; the clubs of the pathogenic *Actinomyces bovis*, although somewhat different in structure, belong to these involution forms.

A complete study of the work done by previous investigators, particularly that of Lechner-Sandoval (23) and Neukirch (30) is given in the paper by Drechsler (13), who made a thorough study of the morphology of this group of organisms, which, with certain limitations, as was pointed out above, is the most complete work done on this subject. In view of the fact that the work of Drechsler was carried on, to some extent, with the organisms reported by the author and also treated in the following pages of this paper, and since it was almost impossible at present to carry on any extensive morphological studies to such a degree as carried out by Drechsler, the morphology of the different species studied will not be taken up to any large extent in this paper. It may though not be out of place to give the summary of Drechsler's work (page 161).

1. The vegetative thallus of Actinomyces consists of a mycelium composed of profusely branching hyphae, the terminal growing portions of which are densely filled with protoplasm. Toward the center of the thallus the vacuoles increase in size and may be associated with the presence of metachromatic granules, the latter having in common nothing with bacterial endospores or "micrococci," for which they were mistaken by early observers.

2. The vegetative mycelium attains an extent incomparably greater than the branching figures recorded for bacteria of the acid-fast group, and the hyphae lack the uniformity in diameter generally characteristic of the Schizomycetes.

3. The aerial mycelium produced on suitable substrata by most species occurs usually in the form of a mat of discrete fructifications; but in other species these fructifications are frequently combined to form numerous and peculiar erect Isarioid sporodochia.

4. In any case each individual fructification represents a well characterized sporogenous apparatus, consisting of a sterile axial filament bearing branches in an open racemose or dense capitate arrangement. The primary branches may function directly as sporogenous hyphae, or may proliferate branches of the second and of higher orders, sporogenesis in the latter case being confined to the terminal elements, the hyphal portions below the points of attachment of branches remaining sterile.

5. Two tendencies in the development of fructifications are recognizable: one leading to an erect dendroidal type, in which successively proliferated fertile elements undergo processes of sporogenesis in continuous sequence; and the other leading to a prostrate racemose type, in which sporogenesis is delayed in the older branches until the younger branches have also attained their final extension. The majority of species show these tendencies combined in different ways.

6. The sporogenous hyphae of most species are coiled in peculiar spirals, sometimes resembling the spores of the hyphomoycetous genus *Helicoon*. These spirals exhibit pronounced specific characteristics in the number, diameter, and obliquity of their turns, and especially in the direction of rotation (whether dextrorose or sinistrorose).

7. Sporogenesis, where it can be followed, begins at the tips of the fertile branches and proceeds basipetally. In the larger number of species the process involves the insertion of septa which, in certain cases, are relatively very massive, and in others so thin as to be barely discernible. The disposition of these septa, while the delimited spores undergo maturation processes, varies with the species: (a) they may remain more or less unaltered; (b) they may suffer a median split, the two resulting halves being then separated as the result of the longitudinal contraction of the young spores, leaving alternate portions of hyphal walls completely evacuated; or (c) they may first become considerably constricted and subsequently converted into non-stainable isthmuses connecting the mature spores. The apparent absence of septa in the sporogenous hyphae of other forms is perhaps attributable to optical difficulties.

8. Granules are readily differentiated in the spores of many species which possess staining properties and uniformity of size characteristic of nuclei; they generally o singly, but in the larger spores of a few forms are often found occupying diagonally opp positions.

9. As in the vegetative thallus, metachromatic granules occur in the aerial mycel being very rarely found in the spores or sporogenous hyphae, but becoming very abun in degenerate sterile hyphae.

10. The older axial filaments of some species show marked distensions which, in extr cases, result in figures simulating *Leptomitus*. These arise as local distensions at the p of attachment of the more extensive lateral sporogenous processes. Cuneate modificat of the sterile axial filaments below the origins of branches also occur.

11. Curious spherical structures appear regularly in some forms, both in the sterile hyphae, where they may contain either a median septum of a number of peripheral m chromatic granules, and in the sporogenous hyphae, where they are associated with regularly spaced septa.

12. The spores germinate readily in suitable solutions, producing 1–4 germ tubes, approximate number being more or less characteristic of the species.

CULTURAL AND BIOCHEMICAL STUDIES

The importance of using simple media and giving their exact chem composition, in the study of actinomycetes, cannot be overemphasized. merous examples can be cited about conspicuous differences obtained wi slight change in the composition of the medium. The production of pigm which was used to a great extent by previous investigators in classifying tl organisms, is almost entirely dependent upon the composition of the med (as well as upon other factors to a smaller extent); this was one of the c reasons why different investigators described the same organism under dif ent names. If the proper substances are offered in the proper forms, dif ences in concentration of these substances do not affect the growth of the tures to such an extent.

Nocard (31) was the first one to cultivate in 1888 an Actinomyces in p culture. This organism was exclusively aerobic, did not modify the reac of neutral bouillon, even if sugar was added; when kept for 4 months at 4(it could still grow vigorously on fresh media; 10 minutes at 70°C. was suffici to destroy the virulence and vitality of the organism. Bostroem (4) mad exhaustive study on the cultivation of pathogenic actinomycetes. Men should also be made of the work of Gasperini (17), Rossi-Doria (34) and M grave, Clegg and Polk (28). Rossi-Doria (34) and Sanfelice (35) studied pathogenicity of the actinomycetes isolated from different sources and st that some of them proved to be pathogenic. Wright (47), having isolat number of pathogenic forms from men and animals, concluded from the s ilarity in morphology and difficulty of cultivation, that they were all one spe (*A. bovis*), making a rather poor growth on milk, potato and coagulated refusing to grow at room temperature and essentially anaerobic.

The cultural studies of actinomycetes by these and other investigators i limited value due to the fact that only complex organic media were used,

standard in composition. The more recent workers on this group of organisms, namely Krainsky (22), Waksman and Curtis (44) and Conn (10), have introduced synthetic inorganic media and have developed several of these, so that they would allow a characteristic growth which helps to differentiate these organisms. On media, which do not contain favorable nutrients, such as nitrates and ammonium salts as sources of nitrogen and saccharose as a source of carbon, the organisms grow rather slowly and have a tendency to spread, while on media containing more favorable nitrogen (asparagin, peptone, etc.) and carbon sources (dextrose, starch, glycerin, etc.), they have a tendency to pile up and often the growth in the last cases may not be so characteristic. The poor media seem to call forth a typical development of the organisms; these should not be taken to hold true for all of them. Gilbert (19) stated that admission of air, dryness and the presence of carbohydrates in the medium, favor spore formation.

Culture media

The following media have been used for the study of cultural and biochemical characters of the actinomycetes. A number of others, not given here, were tried, but the data were not reported when found uncharacteristic.

1. Synthetic solution (*Czapek's,* modified). K_2HPO_4, 1 gm.; $MgSO_4$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, 0.01 gm.; $NaNO_3$, 2 gm.; saccharose, 30 gm.; (in some instances saccharose was replaced by another carbohydrate, 30 gm. per liter); distilled water, 1000 cc. (a better growth is obtained by most species, when glycerin (30 gm.) is substituted for saccharose).

2. Synthetic agar (based on modified Czapek's solution). Same as above, with the addition of 15 gm. of agar per liter. This medium, together with the two media of Krainsky, was found to give the best results for the study of the morphology and cultural characters of the actinomycetes. Only those organisms that produce invertase make an abundant growth on this medium, but since the invertase-producing species were found to be few in number, the growth is rather scant, particularly on repeated transfer, and for that very reason characteristic, since a good development of the aerial mycelium takes place. The cultures should not be grown on this medium continuously, since they will tend to die out.

3. Dextrose nitrate agar and glycerin nitrate agar. In these either dextrose (30 gm.) or glycerin (30 gm.) is substituted for saccharose in the above medium. These two media will allow a much heavier growth of the organisms to take place, and will also give a characteristic growth of some species, but not all of them.

4. Dextrose agar (*Krainsky's,* p. 688). Dextrose, 10 gm.; K_2HPO_4, 0.5 gm.; asparagin 0.5 gm.; agar, 15 gm.; distilled water, 1000 cc.

5. Calcium malate agar (*Krainsky's,* p. 679) with the addition of glycerin as suggested by Conn (10) (*malate-glycerin agar*). Calcium malate, 10 gm.; NH_4Cl, 0.5 gm.; K_2HPO_4, 0.5 gm.; glycerin, 10 gm.; agar, 15 gm.; distilled water, 1000 cc.; reaction adjusted by use of NaOH to P_H 7.0.

6. Egg-albumin agar. Dextrose, 10 gm.; K_2HHO_4, 0.5 gm.; $MgSO_4$, 0.2 gm.; $Fe_2(SO_4)_3$, trace; egg albumin, 0.15 gm.; agar, 15 gm.; distilled water, 1000 cc. The egg albumin is dissolved in N/10 NaOH until neutral to phenolphthalein, then added to the warm medium.

7. Glycerin asparaginate agar (Conn (10), p. 13). Dextrose, 1 gm.; glycerin, 10 gm.; sodium asparaginate, 1 gm.; $NH_4H_2PO_4$, 1.5 gm.; $CaCl_2$, 0.1 gm.; $MgSO_4$, 0.2 gm.; KCl, 0.1 gm.; $FeCl_3$, trace; agar, 12 gm.; distilled water, 1000 cc. Reaction adjusted by the addition of 8 cc. N/10 NaOH. The last two media were found to be very good for the isolation of the organisms, but not for cultural studies.

8. Nutrient agar. Peptone, 10 gm.; Liebig's extract, 5 gm.; NaCl, 5 gm.; agar, 20 gm.; distilled water, 1000 cc. Reaction adjusted to P_H 7.0 to 7.2. The addition of 1-2 per cent of glycerin to this agar makes it excellent for the growth of these organisms (can be used for carrying on cultures).

9. Glucose broth. Glucose, 10 gm.; peptone, 10 gm.; Liebig's meat extract, 5 gm.; NaCl, 5 gm.; distilled water, 1000 cc. Adjusted to P_H 7.0 to 7.2.

10. Egg media. Whole egg (unless otherwise stated) mixed, by means of a sterile spatula in a sterile container, tubed into sterile test tubes, coagulated and sterilized at 80 to 85°C. for 1 hour on 3 consecutive days. The addition of glycerin, as in the case of the Lubenau's or Petroff's medium, makes the growth, in some cases, more characteristic. The introduction of gentian violet in Petroff's (32) medium does not interfere with the growth, thus suggesting its use for isolation of pathogenic forms.

11. Loeffler's blood serum, prepared according to the standard formula (three-fourths beef serum and one-fourth glucose bouillon), coagulated and sterilized as egg media.

12. Blood agar. Ten per cent of rabbit blood added to sterile, redissolved and cooled nutrient agar; tubed, slanted or plated, and incubated for 48 hours to insure sterility.

13. Potato plugs, prepared in the usual manner and placed in test tubes, having a piece of glass rod on the bottom.

14. Carrot plugs, same as potato plugs.

15. Starch agar (27). Ten grams of starch were suspended in 800 cc. of water and boiled until the volume was reduced to 500 cc.; 500 cc. of the medium having the following composition: K_2HPO_4, 1 gm.; $MgSO_4$, 1 gm.; NaCl, 1 gm.; $(NH_4)_2SO_4$, 2 gm.; $CaCO_3$, 3 gm.; agar, 10 gm.; tap water, 500 cc. were added and the medium completed as usual.

16. Cellulose agar. To 500 cc. of cellulose solution prepared by the method of McBeth and Scales (27) was added 500 cc. of a medium having the same composition as in 15, omitting the carbohydrate.

17. Skimmed milk. Fresh milk, separated and sterilized at 10 pounds for 30 minutes or on 3 consecutive days in flowing steam. Brom cresol purple was used for the study of the changes in reaction, according to Clark and Lubs (6).

18. Gelatin. Fifteen per cent of gold-label gelatin in distilled water. Reac-

medium, while others limit their growth to the surface. This would seem to indicate that the actinomycetes are not strict anaerobes, but some may be able to thrive under semi-anaerobic conditions.

Character of growth

Nadson (29) already pointed out that the term colony is used incorrectly in designating a mass of growth of an Actinomyces, since it is not true to nature to call a mass of mycelium developing out of a spore a colony, as in the sense of a bacterial colony. In the case of bacteria, we have colonies of groups of organisms, while in the case of actinomycetes, we have only one organism developing an extensive mycelium, therefore the term "pseudocolony" should rather be used. In the following pages the so-called colony, the growth below and above the substratum, will be termed "growth," "mass of growth" or "colony," as the term is commonly applied. In designating the amount of growth as well as other activities of the organisms, 1 means faint or scant, 2 fair, 3 good, 4 very good, and 5 excellent.

The single-celled Actinomyces colony is usually round and develops in the form of a semi-circle into the medium; most of the species form elastic-like colonies, which cannot be easily broken and are lifted by the needle out of the agar; the surface is usually dry and often presents a conical appearance, particularly when covered with the aerial mycelium; growing hyphae developing into the medium present, on detailed study (with magnifying glass), the typical character of the development of a mold, particularly in young cultures. The surface is usually covered with an aerial mycelium, either cottony, powdery, or smooth, either covering the entire surface or only in patches. The type of mycelium as well as spore formation is shown in the plates. Several species, such as A. verne and A. bobili never produce any true aerial mycelium, but form a heavy folded or lichnoid growth, which changes very little with age; other forms, such as A. lavendulae, A. fradii, A. albosporeus may lose their ability to form the aerial mycelium when grown continuously on Medium No. 2, which is rather poor for making a good growth; when transferred upon other media they regain this property; while still others, notably A. halstedii, may lose entirely, upon continued cultivation on artificial culture media, their power to produce any aerial mycelium at all, and all attempts to bring it back to the original condition have failed so far. It should be noted here that all the cultures, unless otherwise stated, were incubated at 25°C. for 15 days.

Physiological action upon the media

Certain physiological effects of the growth of the actinomycetes upon media that were found to be significant and would help in separating the different organisms, are given. But one should always keep in mind the great variability of these organisms; certain characters may be changed on continued cul-

tivation on artificial culture media; some of these may even be characteristic of the species, such as the production of aerial mycelium, color of the potato plug, or pigmentation of gelatin. These characters can, in many cases, be regained, when the organism is grown on natural substrata and on favorable culture media; if not, one character should not be of sufficient importance to separate the strain into a different group; attention will be called to these facts later. This is the reason why it is so hard to identify an Actinomyces from a description, when not sufficient information is given.

The data observed in repeated series of culture are as follows: changes produced on milk, gelatin, egg and serum media, including liquefaction of solid media, changes in reaction (hydrogen-ion concentration) and pigment production. Studies were also made of the utilization of carbon and nitrogen compounds, proteolytic and diastatic action, growth on cellulose, reduction of nitrates and production of enzymes for all or few species. Odor production was made use of in the first paper, but it does not seem to be characteristic merely of a species, but of the whole group, and it is probably more of a qualitative rather than quantitative nature, depending on many factors.

Milk. The action upon milk seems to be quite characteristic of the species, although too many variations will be found here. The organism may coagulate the milk with a different speed. An organism may hydrolyse (clear) the milk, without any previous coagulation; coagulation may take place here too, but the coagulum is either too soft to be detected easily, or the clotting takes place in the form of fine flakes falling to the bottom which may easily be overlooked, as was found in several instances. Other organisms may not produce any visible changes in the milk. The proteolytic action was followed by the determination of amino-nitrogen of the substratum, using the micro-apparatus of Van Slyke. The change of reaction was studied by means of brom cresol purple, which is much superior for this work to litmus or azolitmin; this was prepared and added to the milk according to the method outlined by Clark and Lubs (5). The milk cultures were all grown at 37°. In designating the proteolytic action on milk, the following terms were used: very faint, when less than 10 per cent of the protein and other nitrogen of the milk has been transformed into amino-nitrogen in 40 days; faint or scant, 10–20 per cent; fair, 20–30 per cent; good, 30–40 per cent; very good, 40–50 per cent; excellent, above 50 per cent. In designating the final reaction of the milk, 0 means unchanged, 1 faintly alkaline, 2 fairly alkaline, 3 distinctly alkaline and 4 and 5 strongly or most alkaline with the particular indicator. Peptonization refers to the digestion of the clot previously formed, while hydrolysis refers to the clearing of the milk, without any visible clot formation. The amino and ammonia nitrogen were determined, in the case of coagulation of the milk, only on the liquefied portion, unless otherwise specified. Some organisms digest the precipitated casein so thoroughy that on the addition of acetic acid no precipitate is obtained, as in the case with *A. griseus, A.* 206, *A. poolensis, A. flavovirens, A. chromogenus* 205, and *A. griseolus.* The growth on the milk

was determined only at 25°, since very little of the growth itself was produced at 37°.

Gelatin. The liquefaction of gelatin cannot be used as a distinguishing character of the actinomycetes, because they all, with very few exceptions (*A. asteroides*), liquefy gelatin. The rapidity of liquefaction and the production of a soluble pigment are characteristic. Even here we find certain limitations: the temperature of incubation, length of cultivation of the organism and media on which it was grown previously, affect to some extent the rapidity of liquefaction, but, allowing for this, we find this characteristic of most species. The production of a brown pigment in the liquefied portion which spreads often into the unliquefied portion of the gelatin is characteristic of the species, with very few exceptions, when this ability is lost on continued cultivation upon artificial culture media. Few organisms produce a faint yellow to golden pigment; a green and a blue pigment were once obtained, but those organisms were lost during the course of the work.

In nearly all cases a 15 per cent solution of gelatin in distilled water was used, the reaction usually left unchanged. In one series of comparative culture, 1 per cent of starch was added to the gelatin, to study the effect of available carbohydrates upon the proteolytic action upon gelatin. The temperature of incubation is very important, since changes in temperature (between 18° and 25°C.) may affect the rapidity of liquefaction by the different organisms, affecting the rate of growth and thus affecting the comparative value of the data obtained. The cultures were grown either in Petri dishes or in tubes of equal diameter, and either the width of the liquefied zone in the dish or the height of the liquefied portion in the tube was measured at the end of a definite period (15–30 days at 18°). The liquefaction is often designated as rapid, medium and slow, depending on the width of the zone or height of the liquefied portion in the tube. The gelatin is either rapidly peptonized and a freely mobile fluid results (*A. albosporeus, A. griseus, A. flavorirens*, etc.), or gradually softened, the medium becoming thickly viscid (*Actinomyces* 205, *A. aureus*), which is true of the rather weakly proteolytic organisms.

Hydrolysis of starch. Starch was found to be, as will be shown later, one of the best sources of energy for the actinomycetes. The hydrolysis of starch was studied by three different methods. The saccharose in the synthetic solution was replaced by 1 per cent starch, the medium distributed in 50-cc. portions in flasks, sterilized and inoculated; at the end of 7, 14, 21 and 28 days, the solution was tested with iodine solution for the presence of starch. The second method was the starch plate, the formula for the medium having been given above; at the end of a definite period of time, a solution of iodine in potassium iodide was poured over the plate, and the width of the clear zone measured. The third method consisted in placing the synthetic solution containing 1 per cent starch, in place of saccharose, in test tubes of equal diameter, 10 cc. to a tube, the line between the hydrolized and unhydrolized starch being readily recognized. At the end of a definite period of incubation, the height

◄of the starch in the inoculated tube was compared with that of the uninoculated tube serving as control, this height measured serving as an indication of the rapidity of hydrolysis of starch or diastatic action. A portion of the clear liquid was withdrawn with a pipette and analyzed for starch and sugar. Most actinomycetes possess a very strong diastatic power both amyloclastic and saccharogenic, hydrolyzing the starch to sugar; in some cases the hydrolysis is incomplete, since only the erythroreaction was obtained. Foulerton and Jones (16) reported that the 10 pathogenic species studied did not exhibit any diastatic action upon starch. This could be confirmed only in the case of *A. asteroides*, while the *A. hominis*, *A. madurae* and *A. bovis* exhibited marked diastatic action upon starch much similar to the saprophytic species. This difference may be due perhaps to the continued cultivation on artificial culture media. In describing the diastatic action of an organism, faint (1) designates a clear zone obtained on the starch plate in 12–15 days at 25°, 3–4 mm. wide; fair (2) 5–8 mm. wide; good (3) 10–15 mm.; very good (4) 15 mm. and more.

Action on cellulose. A number of methods were used for the study of the action of actinomycetes upon cellulose, but none of them was found satisfactory, some organisms giving a bettter reaction on one medium, and others on another. The plate method allowed a good growth of most organisms, and clear zones were obtained with several species, namely *A. violaceus-ruber*, *A. bobili*, *A. exfoliatus* and *A. albus*. Krainsky (22) objected to the use of this method, stating that the clear zone may be due to the solubility of the CaCO₃ or the phosphate through the action of the actinomycetes. It is true that this medium is not so suitable for the study of actinomycetes as for the study of cellulose-splitting bacteria, because the former will grow on this medium, even without the cellulose, on the agar alone. The methods suggested by Krainsky are not much better, since most species refuse to grow on his media at all. The ability to grow on cellulose was demonstrated for several cultures, by merely inserting a piece of filter paper or adding some reprecipitated cellulose to the synthetic solution placed in tubes, then sterilizing and inoculating. The interesting part of it is that few cultures that seemed to have attacked cellulose by one method did not do so by the others; this may be explained by the variation in the composition of the media.

Pigment formation. A number of pigments are produced by actinomycetes. Some of them are insoluble in water, remain in the cells and color the colony or mycelium, while others are soluble and diffuse into the solution or agar, the latter media being more favorable for the pigment production. The complex organic media, such as glucose broth and nutrient agar, are not favorable for the pigment formation. The production of a brown pigment chiefly on organic media which was thought to be characteristic of the *chromogenus* species is the property of several species. It was pointed out in a previous investigation by the author and Curtis (44), as well as by Krainsky (22), that there are many forms which are characterized by the production of a brown to dark

brown pigment on protein media; these species usually show also the chinon reaction on gelatin. Krainsky found that *A. flavo-chromogenus* produced the strongest chinon reaction; this species was not isolated by the writer, but *A. chromogenus* 205 shows the strongest reaction in our series. Gelatin is at first liquefied, then a chinon-gelatin compound is formed which is insoluble. This was not observed with the other chromogenus species. *A. aureus* and *A. lavendulae* would also belong to the chromogenus types, since they produce the same characteristic brown pigment on protein media and attack proteins rather slowly. *A. bobili*, *A. ruber*, *A. flavus* and a few others also produce brown pigments on gelatin and other protein media, but they liquefy the gelatin rapidly.

All the so-called chromogenus species color potato black; this pigment production is ascribed to the enzyme tyrosinase. On incubating all the organisms on the tyrosin agar plate, out of 10–15 species producing brown pigments on proteins and potato plug, only some strains of *A. scabies* formed a dark brown spreading pigment and *A. chromogenus* 205 formed a lighter brown pigment, thus showing that the production of a brown pigment alone on gelatin, potato or other media is no sufficient proof of the identity of the organism.

Beijerinck (3) has shown that by symbiotic action of an Actinomyces with a common soil bacterium, tyrosin in an agar plate culture is oxidized to melanin which appears as black spots on the culture plate. Neither organism alone oxidized tyrosin to the same stage. Other species of Actinomyces produce blue, red or yellow pigments, the simultaneous presence of certain varieties of hay bacteria being favorable in the case of blue and red. Dextrose, malates and nitrates form the chromogeneous food in this case instead of tyrosin. It is considered that the Actinomyces produces homogentisic acid from tyrosin, and that the bacterium oxidizes this acid to melanin. The fact should be kept in mind that gelatin does not contain the amino acid tyrosin, and, since most Actinomyces that produce brown pigments on other media, produce it also on gelatin, we must conclude that the brown pigment is not always due to the production of dark substances (melanins) from tyrosin by the action of a tyrosinase. There seem to be two types of brown pigments: one produced by several species on protein media including gelatin and some pure amino acids, plant media (potatoes, carrots), and synthetic media, and another produced on protein media by organisms which produce dark pigments from tyrosin, as in the case of *A. scabies* and *Actinomyces* 205. Of the soluble pigments only one was studied, namely the red and blue pigment of *A. violaceus-ruber*, which is very interesting. This pigment consists of two or more pigments, which have been definitely demonstrated. One of these pigments acts as an indicator, which is red in acid media and blue in alkaline, the change taking place at about P_H 7.6. On synthetic agar, the organism produces at first a red pigment, since that medium is acid to the indicator (P_H 7.0); with the growth of the organism, the medium changes to alkaline and the pigment turns blue.

Reaction. The methods of study of fermentation of carbohydrates used in the separation of certain bacteria are inapplicable to the actinomycetes: they do not produce any gas and the change of reaction depends more on the source of nitrogen than that of carbon. The reaction was determined in all cases by means of the colorimetric method, the sulfonephthalein series of indicators being used, as recommended by Clark and Lubs (5).

Reduction of nitrates. The reduction of nitrates is a property of nearly all the actinomycetes, differing in a qualitative rather than in a quantitative way, the amount of reduction depending on the source of carbohydrate present in the medium. NaNO₃ was used invariably as the source of nitrate and the amount of nitrite produced was determined by a mixture of sulfonilic acid dissolved in 33 per cent acetic acid and α-naphtylamin, as usually given in the text books. It is characteristic that the reduction of the nitrates took place only to nitrites and not to ammonia or atmospheric nitrogen, at least the latter two have never been demonstrated. Krainsky (22) has already called attention to the fact that, although an active reduction to nitrites was shown only for a few species, many others do it, but the amount of reduction is so small that the nitrite is used up by the organism as soon as formed. The author (43) has shown elsewhere that certain organisms, such as *A. violaceus-ruber*, reduce nitrates very actively with all sources of carbon, while others do not show any reduction with most carbon compounds and only small quantities with others. The members of the first group usually assimilate nitrites very readily, while the members of the other may not. But there is no sharp line of demarkation between these two groups, with many organisms coming intermediate. It should be noted here that not the actual formation but the accumulation of nitrites was necessarily measured.

Proteolytic action. The proteolytic action of the actinomycete was measured in most instances by the amount of amino nitrogen or ammonia accumulated as a result of the action of the organism upon a given protein. The amino nitrogen was measured by the micro-apparatus of Van Slyke (38) and the ammonia by the Folin aeration method (15); in this way we can follow the splitting of the protein. Of course, we do not get the total amino nitrogen produced, since some of it is used up by the organisms and some transformed into other nitrogen compounds, such as ammonia. In determining the amino nitrogen present, when an amino acid is offered as a source of nitrogen, we can follow the process of utilization of the given source of nitrogen. The value of the determination of ammonia as an index of proteolytic action has been greatly overestimated by many bacteriologists, since it is probably a waste product in protein metabolism and depends on different factors. This has been discussed by the writer elsewhere (42). In differentiating the utilization of a definite compound from proteolytic action, the following points should be kept in mind: the amount of growth produced was taken as a measure of utilization, while the splitting of the protein, as measured by the amino and ammonia nitrogen (residual) was taken as a measure of proteolytic action.

Enzyme production. The following enzymes were studied: rennet-like enzymes, protease, invertase, diastase, cellase (or cytase) and tyrosinase.

Some of the data on the metabolism of this group of organisms that may have a direct bearing upon the separation of the different members of the group will be given at the end of this paper, under "Cultural and biochemical studies."

DESCRIPTION OF THE SPECIES

Actinomyces alboflavus Waksman and Curtis

I. MORPHOLOGY.
    1. Spirals.
        Synthetic agar: None; mycelium is straight, branching.
        Calcium malate agar: Straight, branching mycelium, with very little tendency
          to form spirals.
    2. Conidia.
        Synthetic agar: Very few oval-shaped conidia observed.

II. CULTURAL CHARACTERISTICS.
    1. Synthetic agar.
        Growth: Glossy, spreading, colorless at first, later becoming yellowish.
        Aerial mycelium: White powdery, with yellow tinge; property nearly all lost
          with the growth of culture on artificial media.
        Soluble pigment: None.
    2. Calcium malate-glycerin agar.
        Growth: Glossy, restricted, limited to surface, light pinkish-cinnamon color
          (Rdg. XXIX, 15″-d).
        Aerial mycelium: None in 15 days; faint powdery white sprinkling in 30 days.
        Soluble pigment: None.
    3. Glucose agar.
        Growth: Restricted surface growth, much folded; color creamy, with sulfur-
          yellow (Rdg. V, 25-f) tinge of surface.
        Aerial mycelium: None.
        Soluble pigment: None.
    4. Nutrient agar.
        Growth: Restricted, cream-colored.
        Aerial mycelium: None.
        Soluble pigment: None.
    5. Blood serum, 37°. No growth.
    6. Egg-media, 37°. No growth.
    7. Starch agar, 25°, 16 days.
        Growth: Thin, yellowish colored, spreading surface growth.
        Aerial mycelium: None.
        Enzymatic zone: 12–14 mm. wide, hydrolysis incomplete.
    8. Potato plug.
        Growth: Moist, wrinkled, cream-colored growth is produced in 4 days.
        Aerial mycelium: None, sometimes white mycelium is produced.
        Color of plug: White.
    9. Carrot, 25°, 22 days.
        Growth: Numerous cream-colored colonies.
        Aerial mycelium: Thin, powdery, white, all over surface, developing in 15 days.
        Color of plug: Unchanged.

2. Calcium malate-glycerin agar.

Growth: Thin spreading growth, with smooth entire edge; rose colored, with wide colorless margin.

Aerial mycelium: White, powdery, only in center of growth.

Soluble pigment: None.

3. Glucose agar.

Growth: Spreading surface growth, wrinkled and radiating toward periphery edge entire; color Acajou red (Red. XIII, 1'–i) with white colorless margin.

Aerial mycelium: None in 15 days; white patches appear in 30 days.

Soluble pigment: None.

4. Nutrient agar.

Growth: Minute, cream-colored colonies.

Aerial mycelium: None. ·

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Restricted, pink-colored.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, spreading, wrinkled, gray growth in 6 days; with age of culture (30 days), a brownish tinge develops.

Aerial mycelium: None.

Soluble pigment: None.

7. Starch plate, 25°, 12 days.

Growth: Thin, spreading, transparent, with red tinge.

Aerial mycelium: None.

Ensymatic zone: Broad (20 mm. and more).

8. Potato plug.

Growth: Thin, spreading, slightly wrinkled in center; color of growth gray at first, later becoming brown and gray, with greenish tinge (30 days).

Aerial mycelium: None, sometimes white mycelium is produced.

Color of plug: Unchanged.

9. Carrot, 25°, 22 days.

Growth: Scant, restricted, folded, cream-colored, edges turning pink.

Aerial mycelium: None.

Color of plug: Unchanged.

10. Gelatin, 18°, 15 days.

Growth: Yellow, later changing to red color, with hyaline margin; mass of colorless flakes dropping to bottom of liquefied portion.

Aerial mycelium: None, sometimes gray patch.

Soluble pigment: None.

Liquefaction: Rapid (2–3 cm. of depth of tube liquefied in 35 days).

11. Synthetic solution.

Growth: Small pinkish flaky colonies.

Aerial mycelium: Rose-colored.

Soluble pigment: None.

12. Milk. No visible action on milk at both 25° and 37°. ·

Growth (25°): Pinkish scant surface ring.

Coagulation: None.

Hydrolysis: None.

Change of reaction: Unchanged.

13. Glucose broth, 25°, 12 days.
> *Growth:* Pinkish ring on surface in contact with glass.
> *Aerial mycelium:* White, scant.
> *Soluble pigment:* None.

14. Utilization of different carbon compounds.

Arabinose.......... 0	Dextrose............ 3	Lactose............. 2
Glycerin........... 1	Saccharose..........1-2	Maltose............. 1
Cellulose........... 0	Mannite............ 4	Starch.............. 4
Organic acids........ 1		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with different sources of carbon.
2. *Proteolytic action:* Faint on milk; good on gelatin, but only fair, when 1 per cent of starch is added; good on glucose broth.
3. *Change in reaction:* Usually becomes slightly alkaline with NaNO₃ as source of nitrogen with different carbon compounds; distinctly alkaline in acid gelatin, but only faintly alkaline when 1 per cent of starch is present; faint acidity in alkaline glucose broth and no change of reaction in milk.
4. *Inversion of sugar:* Positive, often negative.
5. *Diastatic action:* Fair; 1 per cent starch not used up in 14 days; very good on starch plate, zone 20 mm. and more wide.
6. *Growth on cellulose:* None to very scant.

Hab. Upland California soil.

Actinomyces albus Krainsky, 1914, p. 683, emend. Waksman and Curtis

This organism resembles in certain respects the one described by Krainsky and before by others, although it is doubtful, whether it is the same organism as the one that Krainsky described.

I. MORPHOLOGY.

1. Spirals.
> *Synthetic agar:* None; straight branched mycelium. A few short, closed spirals are found on the glycerin-synthetic agar.
2. Conidia.
> *Synthetic agar:* Spherical and oval, 1.2 to 1.6 x 1.1 to 1.4 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar:
> *Growth:* Abundant, spreading, grayish color.
> *Aerial mycelium:* White, covering all growth, produced in 4–5 days.
> *Soluble pigment:* None.
2. Calcium malate-glycerin agar.
> *Growth:* Thin, penetrating deep into the medium, edge myceloid, gray colored.
> *Aerial mycelium:* Abundant, all over surface, pale mouse gray (Rdg. LI, 15′′′′′–d) with a large white edge.
> *Soluble pigment:* None.

3. Glucose agar.

   Growth: Thick, surface growth slightly elevated in center, penetrating to some extent into medium; edge, myceloid, radial lines from center to periphery; color gray with yellowish center.

   Aerial mycelium: Powdery, all over growth, except narrow edge, pale mouse gray color (Rdg. LI, 15''''-d).

   Soluble pigment: None.

4. Nutrient agar.

   Growth: Glossy, cream-colored, spreading.

   Aerial mycelium: Few white patches.

   Soluble pigment: None.

5. Blood agar, 37°, 15 days.

   Growth: Green colored, restricted, wrinkled.

   Aerial mycelium: White, often none at all.

   Soluble pigment: None.

   Hemolysis: None.

6. Blood serum, 37°.

   Growth: Thin, cream-colored smear appears early (4 days) and remains unchanged.

   Aerial mycelium: None.

   Soluble pigment: None.

   Liquefaction: None.

7. Egg-media, 37°.

   Growth: Thin, spreading, wrinkled, of a cream color.

   Aerial mycelium: None.

   Soluble pigment: None.

8. Starch plate, 15 days.

   Growth: Thin, spreading, transparent.

   Aerial mycelium: None.

   Soluble pigment: None.

   Ensymatic zone: Broad (10–15 mm.).

9. Potato plug.

   Growth: Abundant, much wrinkled, cream-colored, with a faint greenish tinge developing in 15 days.

   Aerial mycelium: Thin, white, only covering edge of growth.

   Color of plug: Purplish with age.

10. Carrot, 25°, 22 days.

    Growth: Abundant, spreading, much folded, cream-colored.

    Aerial mycelium: Very thin, white, powdery.

    Color of plug: Unchanged.

11. Gelatin, 18°C.

    Growth: Small, cream-colored masses on surface and throughout the liquefied portion.

    Aerial mycelium: White patches.

    Soluble pigment: Brown at first, but on continued cultivation on artificial culture media, power of pigment production is lost.

    Liquefaction: Medium (1 cm. of depth of tube in 35 days).

12. Synthetic solution.

    Growth: Small, white colonies on glass of tube, which may also form a surface pellicle.

    Aerial mycelium: None or thin white.

    Soluble pigment: None.

13. Milk, 37°.
> *Growth* (25°): Brownish surface ring.
> *Coagulation:* None.
> *Hydrolysis:* Complete in 20 days, leaving clear solution.
> *Change of reaction:* Strongly alkaline (5).

14. Glucose broth, 25°, 12 days.
> *Growth:* White ring on surface, in contact with glass; also abundant, colorless flaky mass on bottom of tube.
> *Aerial mycelium:* None to scant white.
> *Soluble pigment:* None.

15. Utilization of different carbon compounds.

Arabinose 2	*Dextrose* 3	*Lactose* 3
Glycerin 3	*Saccharose* 2–3	*Maltose* 3
Cellulose 0	*Mannite* 3	*Starch* 3
Organic acids .... 1–2 (lactate)		

16. Utilization of different nitrogen compounds.

Ammonium sulfate 0–1	*Ammonium carbonate* 0
Sodium nitrite 1	*Acetamide* 1
Sodium nitrate 1–5	*Leucin* 3
Glycocoll 3–4	*Casein* 2–3
Asparagin 2	*Fibrin* 3
Egg-albumin 3–4	*Urea* 1
Peptone 2–4	

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good, with starch as a source of carbon; none or very little, with saccharose and glycerin.
2. *Proteolytic action:* Good in milk; faint on gelatin, with practically none in the presence of starch.
3. *Change of reaction:* With NaNO₃ as a source of nitrogen and different carbon compounds, there may be no change, slight acidity or slight alkalinity; strongly alkaline in milk; distinctly alkaline in acid gelatin, in absence of available carbohydrates, only faintly alkaline in presence of starch; acid in alkaline glucose broth (P_H 7.9 changed to 7.1).
4. *Inversion of sugar:* None.
5. *Diastatic action:* Good, 1 per cent starch being used up in 14 days; good on plate, zone 10–15 mm. wide in 15 days.
6. *Growth on cellulose:* No growth in solution with strips of paper as source of carbon; good growth on cellulose plate, with clear zone (1 mm.) around colony.

Hab. This organism was isolated from New Jersey orchard and garden, Colorado, Oregon and California adobe soils.

Actinomyces asteroides (Eppinger) Gasperini (Syn. *Streptothrix eppingeri* Rossi-Doria)

I. MORPHOLOGY.

1. Spirals.
> No true spirals observed; the straight, fine mycelium may show the wavy effect or a few spirals may be produced.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
   Growth: Thin, spreading, penetrating deep into the medium, orange colored.
   Aerial mycelium: None.
   Soluble pigment: None.
2. Calcium malate-glycerin agar and glucose agar, 25°, 12 days.
   Growth: Folded, irregular, light orange, chiefly on surface of medium.
   Aerial mycelium: Faint white on calcium malate, none on glucose agar.
   Soluble pigment: None.
3. Nutrient agar, 25°, 15 days.
   Growth: Much folded, chiefly on surface, at first light yellow, later turning deeper yellow, yellowish red to almost orange colored.
   Aerial mycelium: None or traces of white.
   Soluble pigment: None.
4. Blood agar, 37°, 15 days.
   Growth: Thin, brownish smear in 24–48 hours.
   Aerial mycelium: Gray, appearing in 8–10 days.
   Soluble pigment: None.
   Hemolysis: None.
5. Blood serum, 37°.
   Growth: None at first, later (15 days) thin, white smear is formed; often a good growth is formed in 2–3 days.
   Aerial mycelium: None.
   Soluble pigment: None.
   Liquefaction: None.
6. Egg-media, 37°.
   Growth: Thin, faintly wrinkled, yellowish growth.
   Aerial mycelium: Thin, white, all over growth.
   Soluble pigment: None.
7. Starch plate, 25°, 15 days.
   Growth: Restricted, scant, orange-colored.
   Aerial mycelium: Thin, white.
   Enzymatic zone: None.
8. Potato plug.
   Growth: Much wrinkled, whitish at first, then yellow colored to almost brick red.
   Aerial mycelium: None or fine powdery efflorescence.
   Color of plug: Unchanged.
9. Carrot, 25°, 22 days.
   Growth: At first (7 days), cream-colored, much folded, restricted growth, later turning orange-colored.
   Aerial mycelium: None.
   Color of plug: Unchanged.
10. Gelatin, 18°, 30 days; 37°, 10 days.
    Growth: Yellowish.
    Aerial mycelium: None.
    Soluble pigment: None.
    Liquefaction: None.
11. Synthetic solution.
    Growth: Pinkish flaky growth on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.

12. Milk, 37°, 20 days. Orange colored ring in contact with glass of tube, no visible transformation of the milk.

Coagulation: None.

Peptonization: None.

Hydrolysis: None.

Change of reaction: Unchanged.

13. Glucose broth, 25°, 12 days.

Growth: Thin, yellowish pellicle over entire surface of liquid.

Aerial mycelium: None.

Soluble pigment: None.

14. Utilization of different carbon compounds.

Arabinose......... 0	Dextrose......... 4	Lactose.......... 2
Glycerin.......... 2	Saccharose....... 2–3	Maltose.......... 3
Cellulose.......... 1	Mannite......... 2	Starch........... 1
Organic acids...... 1–3 (lactate)		

15. Utilization of different nitrogen compounds.

Ammonium sulfate............. 0	Ammonium carbonate........ 0
Sodium nitrate................. 0	Acetamide.................. 1
Sodium nitrite................. 1	Leucin..................... 2
Glycocoll..................... 2	Peptone.................... 2
Asparagin.................... 1	Casein..................... 1–2
Egg-albumin.................. 3	Fibrin..................... 1

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair to excellent, depending on source of carbon; the better the carbon source is for the growth of the organism, the larger are the amounts of nitrites accumulated.

2. *Proteolytic action:* Very faint on milk, gelatin and most other proteins.

3. *Change of reaction:* Unchanged, acid (mannite, glycerin), or alkaline (organic acids and a few others) with NaNO₃ as source of nitrogen; distinctly acid with the different amino acids and proteins used and glycerin as a source of carbon; faint alkalinity in glucose broth.

4. *Inversion of sugar:* Negative.

5. *Diastatic action:* None, both on plate and tube.

6. *Growth on cellulose:* None.

Hab. Received from Dr. K. F. Meyer, of the Hooper Institute, San Francisco, Cal., who received it from the Pasteur Institute in 1914.

Actinomyces aureus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Numerous long spirals, 17 to 20 x 4 to 5 μ.

Dextrose agar: Few spirals of open type, although most of the aerial mycelium shows the curving tendency; the spirals are sinistrorose, 3.5–4.5 μ in diameter.

2. Conidia.

Synthetic agar: Abundant, spherical to oval, 0.6–1.0 x 0.8–1.4 μ (1.0–1.2 x 1.0–1.5).

Dextrose agar: Elliptical to oval, 0.5–0.8 x 0.8–1.4 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.
   Growth: Spreading, thin, colorless, developing deep into the medium.
   Aerial mycelium: Thin, powdery, at first mouse-gray, later cinnamon
   (Rdg. XLVI, 13'''); ring formation in the aerial mycelium is prominent
   Soluble pigment: None; reverse of growth becomes dark brown.

2. Calcium malate-glycerin agar, 15 days.
   Growth: Spreading, cream-colored, growth penetrating extensively into
   medium; in 35 days, surface growth is almost black.
   Aerial mycelium: Thin powdery, all over surface, leaving only narrow m
   uncovered (portion below surface of medium), of an hair brown
   XLVI, 17'''-i) color, with a white edge.
   Soluble pigment: None.

3. Glucose agar, 15 days.
   Growth: Spreading surface growth, of a light orange color, also penetr
   into medium; center elevated, margin hyaline.
   Aerial mycelium: Powdery, covering nearly all colony, leaving bare edge
   light drab color (Rdg. XLVI, 17'''-b).
   Soluble pigment: None.

4. Nutrient agar, 15 days.
   Growth: Restricted, gray-colored.
   Aerial mycelium: None.
   Soluble pigment: Deep brown, spreading.

5. Blood agar, 37°, 7 days.
   Growth: Dark brown, glossy, restricted, folded.
   Aerial mycelium: None.
   Soluble pigment: Narrow, dark zone around growth.
   Hemolysis: None.

6. Blood serum, 37°.
   Growth: Restricted, cream-colored growth; often no growth at all.
   Aerial mycelium: None.
   Soluble pigment: Dark zone around growth.
   Liquefaction: None.

7. Egg-media, 37°.
   Growth: Thin, wrinkled, brownish-colored growth.
   Aerial mycelium: None.
   Soluble pigment: Narrow, purple zone around growth.

8. Starch plate, 25°, 12 days.
   Growth: Thin, spreading, transparent.
   Aerial mycelium: Buff-colored, all over surface, in zone formation.
   Enzymatic zone: Fair, 8–10 mm.

9. Potato plug, 25°, 10 days.
   Growth: Abundant, much wrinkled, brown-colored, later (15 days) beco
   black with gray margin.
   Aerial mycelium: Thin, white patches, later becoming ash-gray.
   Color of plug: Black.

10. Carrot, 25°, 22 days.
   Growth: Thin, restricted, gray, turning purplish brown.
   Aerial mycelium: None.
   Soluble pigment: Black.

11. Gelatin, 18°, 30 days.

   Growth: Fair, cream-colored, changing to brown, spreading.

   Aerial mycelium: Usually none; white aerial mycelium may be produced at times, particularly in exposed portion of growth.

   Soluble pigment: Brown, spreading into unliquefied portion.

   Liquefaction: At first rapid, later slow.

12. Synthetic solution.

   Growth: Flakes throughout the medium; light powdery colonies on surface.

   Aerial mycelium: Mouse-gray.

   Soluble pigment: None.

13. Milk, 37°. Soluble brown pigment. No visible action on milk at 37°.

   Growth (25°): Black surface ring.

   Coagulation: None, only thickening of milk may often be observed.

   Hydrolysis: In certain cases some digestion is found; in most cases, no visible action upon the milk.

   Change of reaction: Unchanged to faintly alkaline in digested tubes.

14. Glucose broth, 12 days, 25°.

   Growth: Thin brownish ring on surface in contact with glass; flaky mass on bottom of tube.

   Aerial mycelium: None.

   Soluble pigment: Deep brown.

15. Utilization of different carbon compounds.

Arabinose........... 0	*Dextrose*......... 4	*Lactose*.......... 3–4
Glycerin............ 3	*Saccharose*....... 1–2	*Starch*........... 2
Cellulose........... 0	*Organic acids*..... 1	

16. Utilization of different nitrogen compounds (glycerin as source of energy).

Ammonium sulfate............. 1–5	*Ammonium carbonate*.......... 1–2
Sodium nitrite................. 3	*Acetamide*.................... 2
Sodium nitrate................. 3–5	*Leucin*....................... 4–5
Glycocoll..................... 5	*Casein*....................... 4–5
Asparagin.................... 4	*Fibrin*....................... 3–4
Egg-albumin.................. 4–5	*Urea*........................ 1
Peptone...................... 5	

   When glycerin is replaced by dextrose, the ammonium salts and the amides are utilized to a much greater extent.

III. BIOCHEMICAL FEATURES.

   1. *Nitrite formation:* Only with starch and glycerin as sources of carbon, not with saccharose.

   2. *Proteolytic action:* Proteolytically this organism is not very active; it exerts a fair action on milk, gelatin and glucose broth.

   3. *Change of reaction.* Usually slightly alkaline with $NaNO_3$ as a source of nitrogen and different sources of carbon; distinctly alkaline in acid gelatin, both in presence and absence of starch (from P_H 6.2 to P_H 7.6 and 7.4); very distinctly acid in alkaline glucose broth (P_H changed from 7.9 to 5.8 and less).

   4. *Inversion of sugar:* None to positive.

   5. *Diastatic action:* Good; 1 per cent starch nearly all used up in 14 days; fair on plate, zone 8–10 mm. wide.

   6. *Growth on cellulose:* Very good by plate method (no zone formation); but none on paper or precipitated cellulose in solution.

Hab. This is one of the most common organisms, or rather groups of organisms found in the soil; a number of strains have been isolated, which vary in some details but run into one another; the whole group is related to the *chromogenus* species. Isolated from New Jersey garden, orchard, meadow and forest soils, Iowa, Louisiana, North Dakota, Hawaii, Texas, Alaska and Colorado soils.

Actinomyces bobili Waksman and Curtis

I. MORPHOLOGY.

1. Synthetic agar.
    The branching of the hyphae is often so close as to have the appearance of whirls. No spirals.
2. Glycerin-synthetic agar.
    Few close spirals of a dextrorose type.

II. CULTURAL CHACTERISTICS.

1. Synthetic agar.
    Growth: Abundant, glossy, wrinkled, chiefly on surface, elevated, with lichnoid margin, at first coral red (Rdg. XIII, 5′), later becoming Acajou red (Rdg. XIII, 3′).
    Aerial mycelium: None at first; when culture is grown longer on artificial media, scant white mycelium develops.
    Soluble pigment: None.
2. Calcium malate-glycerin agar, 12 days.
    Growth: Spreading, both on surface and into medium; color cinnamon-buff (Rdg. XXIX, 17″-b).
    Aerial mycelium: None.
    Soluble pigment: None.
3. Glucose agar.
    Growth: Restricted, finely wrinkled, chiefly on surface of medium; color coral red (Rdg. XIII, 5′), with hyaline margin.
    Aerial mycelium: None.
    Soluble pigment: None.
4. Nutrient agar.
    Growth: Restricted, glossy, at first gray, later becoming brownish.
    Aerial mycelium: None.
    Soluble pigment: None.
5. Blood serum.
    Growth: Minute, glossy, gray colonies.
    Aerial mycelium: None.
    Soluble pigment: Brown zone around growth.
    Liquefaction: None.
6. Egg-media.
    Growth: Thin, wrinkled, at first (4 days) brown, later (15 days) turning jet black.
    Aerial mycelium: None.
    Soluble pigment: Narrow purple zone around growth.
7. Starch plate, 25°, 16 days.
    Growth: Spreading, pink colored.
    Aerial mycelium: White traces in isolated spots.
    Ensymatic zone: 10–11 mm., hydrolysis incomplete.

8. Potato plug.

> *Growth:* Thin, at first yellowish, later (15 days) turning red; surface dry and wrinkled.
>
> *Aerial mycelium:* Red, with some scant white.
>
> *Color of plug:* At first unchanged, later (15 days) a black zone is formed around the growth.

9. Carrot, 25°, 22 days.

> *Growth:* Abundant, spreading, folded net-like, cream-colored.
>
> *Aerial mycelium:* None.
>
> *Color of plug:* Unchanged.

10. Gelatin, 18°, 30 days.

> *Growth:* Dense cream-colored to brownish.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Brown.
>
> *Liquefaction:* Rapid.

11. Synthetic solution.

> *Growth:* Colorless, with orange center, often pinkish colonies through medium, collecting on bottom of tube.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None to faint yellow.

12. Milk, 37°.

> *Growth:* Dark brown surface ring; brown soluble pigment.
>
> *Coagulation:* None.
>
> *Hydrolysis:* Completed in 15–18 days both at 37° and 25°.
>
> *Change of reaction:* Distinctly alkaline (3).

13. Glucose broth, 25°, 12 days.

> *Growth:* Flakes on bottom and round colonies all throughout medium, chiefly in contact with glass.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None to brownish.

14. Utilization of different carbon compounds.

Arabinose	0	*Dextrose*	2	*Lactose*	3
Glycerin	3	*Saccharose*	1–2	*Maltose*	2
Cellulose	4	*Mannite*	0	*Starch*	3
Organic acids	1–2 (acetate)				

15. Utilization of different nitrogen compounds (glycerin as source of carbon).

Ammonium sulfate	0	*Ammonium carbonate*	0
Sodium nitrite	0	*Acetamide*	1–2
Sodium nitrate	1	*Leucin*	2–3
Glycocoll	2–3	*Casein*	3–4
Asparagin	1	*Fibrin*	3
Egg-albumin	1–3	*Urea*	1
Peptone	2–4		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Very good, with many sources of carbon, although none or mere traces are obtained with saccharose and often with glycerin.

2. *Proteolytic action:* Very good in milk.

3. *Change of reaction:* Usually slight alkalinity, with NaNO₃ as a source of nitrogen.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Very good; 1 per cent starch used up in 7 days; saccharogenic power on plate is not very good, since the hydrolysis of the starch is incomplete in 16 days.

6. *Growth on cellulose:* Good on plate and reprecipitated cellulose, but none on filter paper in solution; clear zone is formed on plate around colony.

Hab. Isolated from New Jersey garden and California adobe soils.

Actinomyces bovis Harz

I. MORPHOLOGY.

1. *Synthetic agar:* Thin, branching hyphae; few open spirals, of a dextrorose type.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Restricted, developing deep into the medium, white, turning yellowish in 10-12 days.

Aerial mycelium: Appears late (20 days), light sulfur-yellow, powdery, covering all growth.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 25°, 20 days.

Growth: Restricted, brownish growth chiefly in the medium.

Aerial mycelium: None.

Soluble pigment: None.

3. Glucose agar, 25°, 20 days.

Growth: Restricted yellowish, later becoming dark, consisting of a mass of small colonies, developing to some extent into the medium.

Aerial mycelium: Thin, sulfur-yellow.

Soluble pigment: None.

4. Nutrient agar.

Growth: Abundant, restricted on surface of medium, at first cream-colored, later becoming fawn-colored, brown, then almost black.

Aerial mycelium: Pale yellow green (Rdg. V, 27 f).

Soluble pigment: None.

5. Blood agar, 37°, 15 days.

Growth: Good, spreading.

Aerial mycelium: None.

Soluble pigment: None.

Hemolysis: Faint at first, 1-2 mm. zone in 15 days.

6. Blood serum, 37°, 15 days.

Growth: Slow, thin, gray growth, later turning yellowish.

Aerial mycelium: Sulfur-yellow.

Soluble pigment: None.

Liquefaction: Medium (12-15 days).

7. Egg media, 37°, 15 days.

Growth: Thin, cream-colored smear.

Aerial mycelium: Thin, white, chiefly over edge of growth.

Soluble pigment: None.

8. Starch plate, 25°, 15 days.

Growth: Growth is of a dirty yellowish color.

Aerial mycelium: None.

Enzymatic zone: Fair, 6-8 mm. wide.

9. Potato plug.

Growth: Abundant, much wrinkled, gray to canary yellow.

Aerial mycelium: Yellow (4 days), turning to characteristic sulfur-yellow (8 days).

Color of plug: Unchanged at first, later (17 days) turning brown.

10. Carrot, 25°, 22 days.

Growth: Fair, restricted, cream-colored, developing a dark reverse, wrinkled.

Aerial mycelium: Sulfur-yellow, powdery, all over surface, without leaving any margin.

Color of plug: Narrow dark zone.

11. Gelatin, 18°, 30 days.

Growth: Gray to brownish.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: Rapid at 37°, slow at 18°.

12. Synthetic solution.

Growth: None. When glycerin is substituted for saccharose, a few colorless flakes are formed on the bottom of the tube.

13. Milk, 37°.

Growth (25°): Thin yellowish surface growth.

Coagulation: 10–12 days.

Peptonisation: Begins soon after coagulation is completed, proceeds somewhat slowly and is all completed in 40 days.

Hydrolysis: Often hydrolysis of the milk, without previous coagulation, may take place.

Change of reaction: Faintly (1) to distinctly alkaline (3).

14. Glucose broth, 25°, 12 days.

Growth: Small, round, flaky, colorless mass on bottom of tube; thin layer of a yellowish tinge on surface.

Aerial mycelium: Sulfur-yellow, scant.

Soluble pigment: None.

15. Utilization of different carbon compounds.

Dextrose............ 2	*Lactose*............ 2	*Glycerin*........... 1
Saccharose.......... 1	*Maltose*............ 1	*Cellulose*........... 0
Organic acids........ 1		

16. Utilization of different nitrogen compounds.

Ammonium sulfate 0	*Ammonium carbonate*.......... 0
Sodium nitrite................. 0	*Acetamide*................... 1
Sodium nitrate................. 1	*Leucin*...................... 2
Glycocoll..................... 2	*Casein*...................... 3–4
Asparagin.................... 2–3	*Peptone*..................... 2
Egg-albumin.................. 3–4	*Fibrin*...................... 2–3
Urea...................... 1	

III. **BIOCHEMICAL FEATURES.**

1. *Nitrite formation:* Excellent with carbon compounds that offer a good source of energy (saccharose, lactose, glycerin).

2. *Proteolytic action:* Good on milk; fair on peptone. casein, fibrin, egg-albumin and gelatin.

3. *Change of reaction:* Faintly alkaline in all cases, when NaNO₃ is the only source of nitrogen; distinctly alkaline with different proteins or amino acids as sources of nitrogen and glycerin as a source of carbon.

4. *Inversion of sugar:* Negative.

5. *Diastatic action:* Fair on plate.

6. *Growth on cellulose:* None or very scant, although good growth was obtained on sterile soil.

Hab. Received from Dr. K. F. Meyer, who had it from the American Museum of Natural History, received from Parke Davis Co., in 1911 (0.4); also received directly from Parke Davis Co., in 1918. The two cultures have shown somewhat different cultural characters; when received, the latter culture did not develop readily any aerial mycelium; but on continued cultivation upon synthetic media, both gave similar cultural and biochemical characters, which varied in their action in quantity rather than in quality.

Actinomyces californicus Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Narrow, long, open spirals, belonging to the corkscrew and sinistrorose type - on the synthetic media.

2. Conidia.

Straight hyphae and spirals break up to form small spherical to oval spores.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Spreading, vinaceous colored (Rdg. XXVII, 1″-d), chiefly in the substratum; surface growth limited only to aerial mycelium.

Aerial mycelium: Powdery, thin light neutral gray (Rdg. LIII, N. G.-C), with distinct zonation.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 15 days.

Growth: Restricted, cream-colored.

Aerial mycelium: Same as on No. 1.

Soluble pigment: None.

3. Glucose agar, 15 days.

Growth: Same as on No. 1, but not spreading so much into the medium.

Aerial mycelium: Same as on No. 1, but more abundant, no zonation, leaving uncovered margin.

Soluble pigment: None.

4. Nutrient agar.

Growth: Thin, restricted, yellowish to creamy in color.

Aerial mycelium: Powdery, covering all the surface of growth, white to cream-colored.

Soluble pigment: None.

5. Blood agar, 37°.

Growth: Gray, with tinge of red, restricted, folded.

Aerial mycelium: None.

Soluble pigment: None, often a dark reverse is obtained.

Hemolysis: None.

6. Blood serum, 37°.

> *Growth:* Cream-colored, spreading.
>
> *Aerial mycelium:* None at first, some white isolated spots appearing in 10–15 days.
>
> *Soluble pigment:* None at first, with a faint brown pigment developing in 10–15 days.
>
> *Liquefaction:* Faint, not increasing with age of culture.

7. Egg-media, 37°, 3 days.

> *Growth:* Cream-colored, spreading.
>
> *Aerial mycelium:* White cottony tufts all over surface.
>
> *Soluble pigment:* None, purplish pigment developing only in 7 days.

8. Starch plate, 25°, 12 days.

> *Growth:* Spreading, central portion pink, with colorless to gray margin.
>
> *Aerial mycelium:* Ash-gray powder all over surface of growth.
>
> *Ensymatic zone:* 4–5 mm. wide.

9. Potato plug.

> *Growth:* Glossy, yellow to red, with age, turning red-brown.
>
> *Aerial mycelium:* None.
>
> *Color of plug:* Unchanged.

10. Carrot, 25°, 22 days.

> *Growth:* Abundant, raised, spreading, covering all plug, of a pinkish shade.
>
> *Aerial mycelium:* Grayish, powdery, abundant, all over surface.
>
> *Color of plug:* Unchanged.

11. Gelatin, 18°, 30 days.

> *Growth:* Gray, moist, abundant surface growth.
>
> *Aerial mycelium:* White, in patches.
>
> *Soluble pigment:* Yellowish green, spreading into the insoluble portion.
>
> *Liquefaction:* Medium, 2 cm. of gelatin in tube liquefied in 30 days.

12. Synthetic solution, 15 days.

> *Growth:* Thin flakes throughout medium.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.
>
> When glycerin is substituted for saccharose, there is a heavy cream-colored pellicle formed, with greenish white aerial mycelium and greenish soluble pigment.

13. Milk, 37°.

> *Growth* (25°): Faint, brownish growth on surface.
>
> *Coagulation:* 6–15 days.
>
> *Peptonisation:* Begins soon after coagulation is complete, advances slowly, coagulum may not all be digested in 40 days, although with an early coagulation digestion is completed in 15–20 days.
>
> *Change of reaction:* Faintly alkaline (1).

14. Glucose broth, 25°, 12 days.

> *Growth:* Solid, cream-colored mass on surface of liquid, with pink tinge on reverse.
>
> *Aerial mycelium:* Cream-colored, all over growth.
>
> *Soluble pigment:* None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Excellent with all sources of carbon; it may be absent in some cultures.

2. *Proteolytic action:* Fair on milk.

3. *Change of reaction:* Usually to alkaline.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Good, starch reduced in 14 days only to erythrodextrin (poor saccharogenic action); only fair diastatic action is obtained on the plate.
6. *Growth on cellulose:* Scant, but definite.
Hab. Isolated from California and cranberry soils.

Actinomyces chromogenus strain 205

I. MORPHOLOGY.
   1. Spirals.
       Numerous, closed spirals, of the fist type produced on the different synthetic media; the side hyphae are often only of a wavy nature.
   2. Conidia.
       Oval to elliptical.

II. CULTURAL CHARACTERISTICS.
   1. Synthetic agar, 15 days.
       Growth: White, spreading deep into the medium.
       Aerial mycelium: Ash-gray with brownish tinge.
       Soluble pigment: None.
   2. Calcium malate-glycerin agar, 15 days.
       Growth: Spreading, colorless on and below surface of medium, edge myceloid.
       Aerial mycelium: Abundant, cottony, all over growth; drops of water on surface; color of mycelium light grayish olive (Rdg. XLVI, 21''''-b), with narrow white margin turning in 30 days dark gray.
       Soluble pigment: None.
   3. Glucose agar, 15 days.
       Growth: Abundant, chiefly on surface of medium, also in substratum; edge entire; color natal brown (Rdg. XL, 13'''-K), changing in 30 days to almost black.
       Aerial mycelium: Abundant, cottony, covering all surface; color white with gray tinge; changing in 30 days to light gray.
       Soluble pigment: Brownish pigment is dissolving into medium.
   4. Nutrient agar, 15 days.
       Growth: Wrinkled, brown colored at first, later turning gray-green.
       Aerial mycelium: White patches.
       Soluble pigment: Brown.
   5. Blood serum, 37°, 7 days.
       Growth: Thin, spreading, brown smear.
       Aerial mycelium: None.
       Soluble pigment: Spreading, dark zone.
       Liquefaction. None.
   6. Egg-media, 37°.
       Growth: Thin, spreading, gray growth, later becoming black (17 days).
       Aerial mycelium: Dark gray.
       Soluble pigment: Spreading black zone.
   7. Potato plug, 25°, 12–15 days.
       Growth: Small, wrinkled, black colonies.
       Aerial mycelium: None.
       Color of plug: All black.

8. Carrot, 15 days.
   Growth: Abundant, spreading, cream-colored.
   Aerial mycelium: White, cottony tufts all over surface; exuded drops are found on surface.
   Color of plug: Dark-brown zone around growth.
9. Starch plate, 15 days.
   Growth: Transparent, spreading.
   Aerial mycelium: Buff-gray in concentric zones.
   Ensymatic zone: 12–15 mm. wide.
10. Gelatin, 18°C.
    Growth: Cream-colored, spreading on surface and side of tube; flaky in the medium.
    Aerial mycelium: Abundant, white, cottony tufts covering all surface growth.
    Soluble pigment: Dark brown, changing to deep olive-green, both in the liquefied and unliquefied portions.
    Liquefaction: Rapid at first, later becoming very slow (5 mm. in 35 days) so that the liquefied portion may solidify again; the liquefaction is more rapid in presence of starch.
    This is the only organism of the whole group which exhibited the quinone action on gelatin; even this organism did not show it always.
11. Synthetic solution.
    Growth: Thin pellicle on surface and colorless flakes throughout the medium.
    Aerial mycelium: Smoke gray; when glycerin is substituted for saccharose, it is yellowish-buff.
    Soluble pigment: None; when glycerin is present, in place of saccharose, it is deep yellow.
12. Milk, 37°. Soluble brown pigment produced.
    Growth (25°): Dark brown ring.
    Coagulation: None at 37°, a clot is often produced at 25°.
    Hydrolysis: If no visible clot is obtained, the milk may undergo hydrolysis in 20 days, often no visible action is observed on the milk.
    Change of reaction: Distinctly alkaline (3).
13. Glucose broth, 25°, 12 days.
    Growth: Thin, brown ring on surface in contact with glass; some flaky colonies on bottom.
    Aerial mycelium: None to grayish brown thin layer.
    Soluble pigment: Dark brown all through liquid.
14. Utilization of different carbon compounds.

Arabinose	0	*Dextrose*	1–2	*Lactose*	2
Glycerin	1–2	*Saccharose*	1–3	*Maltose*	3–4
Cellulose	2	*Mannite*	1	*Starch*	4
Organic acids	2				

III. BIOCHEMICAL FEATURES.
   1. *Nitrite formation:* Very weak, usually none or only faint traces with some sources of carbon.
   2. *Proteolytic action:* Faint in milk; good on gelatin, both in presence and absence of 1 per cent starch.
   3. *Change of reaction:* Usually none, with NaNO₃ as source of nitrogen; often faint acidity (glycerin as source of carbon) or slight alkalinity, in the case of vigorous growth; distinctly alkaline in acid gelatin in presence and absence of starch (P_H changed from 6.2 to 7.4 and 7.8); faintly acid in alkaline glucose broth.

4. *Inversion of sugar:* Positive or negative, the latter is obtained with weak growth.

5. *Diastatic action:* Good on plate.

6. *Production of tyrosinase:* This organism is the only one of the whole group, in addition to *A. scabies,* which produced a dark pigment on the tyrosin agar plate; the pigment was not as deep as that produced by *A. scabies.*

7. *Growth on cellulose:* Very faint and only with certain methods; this organism does not attack cellulose readily.

Hab. Isolated by the writer and Curtis from Maine Aroostook soil.

Actinomyces citreus Krainsky 1914, p. 684, emend. Waksman and Curtis

I. MORPHOLOGY.

    1. Spirals.

        None or very few on synthetic agar and calcium malate agar; long narrow, open spirals on dextrose and starch agar, which are of the dextrorose type.

    2. Conidia.

        Synthetic agar: Spherical to oval, 1.2 to 1.8 x 1.2 to 1.5 μ.

II. CULTURAL CHARACTERISTICS.

    1. Synthetic agar.

        Growth: Abundant, spreading, chiefly on surface also to some extent into medium; raised, wrinkled; color citron-yellow (Rdg. XVI, 23–C).

        Aerial mycelium: Covering all growth, same color (another strain has white aerial mycelium).

        Soluble pigment: None.

    2. Calcium malate-glycerin agar.

        Growth: Good, spreading growth, extending somewhat into the medium; cream-colored with yellowish tinge.

        Aerial mycelium: Extensive, cottony, thick, covering all colony, except narrow edge; white, with mouse-gray tinge, later turning all light gray.

        Soluble pigment: None.

    3. Glucose agar.

        Growth: Very extensive growth, chiefly on surface, penetrating into the medium only to a small extent; center much elevated, edge entire, glossy, color olive-yellow (Rdg. XXX, 23″).

        Aerial mycelium: Cottony, in patches, covering only parts of growth; white colored; in 30 days aerial mycelium is found to be very abundant, thick and pinkish in color.

        Soluble pigment: None.

    4. Nutrient agar.

        Growth: Restricted, cream-colored.

        Aerial mycelium: None.

        Soluble pigment: None.

    5. Blood agar, 37°.

        Growth: Small, restricted, gray colonies.

        Aerial mycelium: None.

        Soluble pigment: None.

        Hemolysis: Begins only late (6 days), advancing slowly; zone of hemolysis 0.5 mm. wide in 21 days.

    6. Blood serum, 37°.

        Growth: Minute cream-colored colonies.

        Aerial mycelium: None.

        Soluble pigment: None.

        Liquefaction: None.

7. Egg-media, 37°.
   Growth: Thin, spreading, wrinkled, cream-colored growth.
   Aerial mycelium: None.
   Soluble pigment: None.
8. Starch plate.
   Growth: Abundant, yellowish-green color, characteristic.
   Aerial mycelium: Abundant, pinkish colored.
   Enzymatic zone: Fair, 5–8 mm. not perfectly cleared.
9. Potato plug.
   Growth: Thin, wrinkled, gray-colored smear.
   Aerial mycelium: Thin, white, but property lost with age, so that on later transfers none is observed.
   Color of plug: Unchanged.
10. Carrot, 25°, 22 days.
    Growth: Abundant, spreading, much folded, brownish colonies.
    Aerial mycelium: Thin, powdery, white in rare patches.
    Color of plug: Unchanged.
11. Gelatin.
    Growth: Yellowish, restricted pellicle, floating on surface or falling to bottom of liquefied pit.
    Aerial mycelium: White, scant.
    Soluble pigment: None.
    Liquefaction: Medium (1 cm. in 35 days).
12. Synthetic solution.
    Growth: Small flakes on bottom.
    Aerial mycelium: None.
    Soluble pigment: None.
    When glycerin is substituted for saccharose, there is formed a white surface pellicle, with a thin white aerial mycelium.
13. Milk, 37°.
    Growth: Cream-colored surface zone and hydrolysis in 15 days at 25°.
    Coagulation: 9–10 days.
    Peptonisation: Rapid; clot all digested in 20 days.
    Hydrolysis: In certain tubes no coagulation takes place, but milk is slowly hydrolized.
    Change of reaction: Distinctly alkaline (3).
14. Glucose broth, 25°, 12 days.
    Growth: Thin, wide, yellowish ring on surface, in contact with glass; often an abundant surface pellicle is formed; few flakes on bottom and throughout medium.
    Aerial mycelium: Scant white over edges of growth.
    Soluble pigment: None to faint yellow.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* None or mere traces of nitrites accumulated, with moist carbon sources.
2. *Proteolytic action:* Very good in milk.
3. *Change of reaction:* Distinct alkalinity in milk; faint acidity in alkaline glucose broth.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Fair on plate.
6. *Growth on cellulose:* None.

Hab. Isolated numerous times from Oregon adobe, New Jersey garden and orchard soils, and identified by Waksman and Curtis as belonging to the above species, although comparison with Krainsky's culture was impossible, as in the case of the other cultures that were believed to be the same as isolated by Krainsky.

Actinomyces diastaticus Krainsky 1914, p. 682, emend. Waksman and Curtis

It is not certain whether or not this organism is exactly the same as that described by Krainsky, since no comparison of cultures was made, it is related culturally and particularly in its diastatic action to the following two organisms or groups: *A. rutgersensis* and *A. lipmanii;* the *A. diastaticus* studied by Krainsky no doubt belongs to one of these three organisms or is at least a closely related form.

I. MORPHOLOGY.
    1. Spirals.
        Usually none on synthetic dextrose and calcium malate agar; sometimes fine, narrow, long spirals may be produced.
    2. Conidia.
        Synthetic agar: Oval shaped, 1.0 to 1-2 x 1.1 to 1.5 μ.

II. CULTURAL CHARACTERISTICS.
    1. Synthetic agar.
        Growth: Spreading, thin, gray, developing deep into medium.
        Aerial mycelium: White, turning drab gray (Rdg. XLVI, 17''''-d).
        Soluble pigment: Brown to dark brown.
    2. Calcium malate-glycerin agar.
        Growth: Spreading on surface and deep into medium, edge glossy, entire; color brown.
        Aerial mycelium: Thin, gray, somewhat powdery, covering whole surface except a narrow margin of colony left uncovered.
        Soluble pigment: None.
    3. Glucose agar.
        Growth: Yellowish, spreading on surface in the form of minute colonies, running into one another, also deep into medium.
        Aerial mycelium: None in 30 days.
        Soluble pigment: None.
    4. Nutrient agar.
        Growth: Cream-colored.
        Aerial mycelium: Thin, white.
        Soluble pigment: None.
    5. Blood agar, 37°.
        Growth: Grayish-brown, restricted, folded.
        Aerial mycelium: None.
        Soluble pigment: Faint brown at first (2 days), later may disappear.
        Hemolysis: Wide clear zone in 10–15 days.
    6. Blood serum, 37°.
        Growth: Restricted, gray colored, appearing late (12–15 days).
        Aerial mycelium: None.
        Soluble pigment: None.
        Liquefaction: Started in 15 days.

7. Egg-media, 37°.

> *Growth:* Very thin, spreading, wrinkled growth, cream-colored, with greenish tinge (5 days).
> *Aerial mycelium:* None.
> *Soluble pigment:* None.

8. Starch plate, 15 days.

> *Growth:* Thin, spreading, colorless.
> *Aerial mycelium:* None.
> *Enzymatic zone:* Very broad, 20 mm., and more.

9. Potato plug.

> *Growth:* Abundant, wrinkled, cream-colored, with greenish tinge, later (15 days) becoming brown.
> *Aerial mycelium:* White, to gray; property is lost with age, so that, on continued cultivation, no aerial mycelium is produced.
> *Color of plug:* Darkened at first; on continued cultivation no change of color of plug is produced.

10. Carrot, 25°, 22 days.

> *Growth:* Abundant, spreading, folded, dark brown, covering all surface of plug
> *Aerial mycelium:* Scant, drab gray.
> *Color of plug:* Brownish, all plug shrivelled up.

11. Gelatin, 18°.

> *Growth:* Small cream-colored flakes dropping to bottom of liquefied portion; some growth on surface.
> *Aerial mycelium:* Gray, covering all growth.
> *Soluble pigment:* None.
> *Liquefaction:* Rapid (3–4 cm. in 35 days).

12. Synthetic solution.

> *Growth:* Flakes throughout medium, often a white surface pellicle is formed.
> *Aerial mycelium:* None or some thin grayish layer.
> *Soluble pigment:* None to yellow, often becoming brownish.

13. Milk, 37°.

> *Growth* (25°): Brownish ring on surface.
> *Coagulation:* 5–7 days.
> *Peptonization:* Begins in 5–7 days, is completed in 25–30 days.
> *Change of reaction:* Faintly alkaline (1).

14. Glucose broth, 25°, 12 days.

> *Growth:* Ring of gray colonies on surface; round, colorless to grayish colonies on bottom of tube.
> *Aerial mycelium:* None.
> *Soluble pigment:* None.

15. Utilization of different carbon compounds.

Arabinose	4	*Dextrose*	4	*Lactose*	4
Glycerin	4	*Saccharose*	1–3	*Maltose*	4
Cellulose	0	*Mannite*	4	*Starch*	4
Organic acids	0–1				

III BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair in presence of most readily available sources of carbon; often none is evident, especially with poor growth.

2. *Proteolytic action:* Good in milk; very good on gelatin, but only faint when 1 per cent of starch is present.

3. *Change of reaction:* Distinct alkalinity, with sodium nitrate as a source of
nitrogen; fairly alkaline in acid gelatin, but only faintly alkaline when 1 per
cent of starch is present; faint alkalinity in milk; faint acidity in alkaline
glucose broth.

4. *Inversion of sugar:* None.

5. *Diastatic action:* Excellent, all starch of a 1 per cent solution used up in 14 days;
excellent on plate, zone more than 20 mm. wide in 15 days.

6. *Growth in cellulose:* None or very scant.

Hab. California sandy loam.

Actinomyces 161

I. MORPHOLOGY.

This species resembles in many respects Krainsky's *A. erythrochromogenus*, but
it does not produce the brown pigment characteristic of the *chromogenus*
species on nutrient agar and gelatin.

Synthetic agar: Numerous open spirals formed as side branches of the main
hyphae. The mycelium is fine, branching. No spirals are observed when
saccharose is replaced by glycerin in the medium.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
   Growth: Spreading with irregular margin, developing deep into the medium;
   color at first white, later turning yellowish; agar around growth has a white
   milky surface.
   Aerial mycelium: Thick, solid, white, all over growth and appears early.
   Soluble pigment: Pomegranate purple (Rdg. XII, 71–i), later turning Bor-
   deaux (Rdg. XII, 71–k) color. On repeated transfers, pigment becomes
   vinaceous colored.

2. Calcium malate-glycerin agar.
   Growth: Restricted, thin on surface, developing well into the medium; sur-
   face smooth, edge lobose; color creamy with shade of pink.
   Aerial mycelium: Thin, covering entire surface, white colored.
   Soluble pigment: None.

3. Glucose agar.
   Growth: Abundant, spreading, cream-colored, later turning brown chiefly on
   surface; center raised; surface presents cracking appearance, edge lobose.
   Aerial mycelium: Thin, white, over entire surface (very thin over margin).
   Soluble pigment: None. Faint brown in 20 days.

4. Nutrient agar.
   Growth: Cream-colored.
   Aerial mycelium: Few white patches.
   Soluble pigment: None.

5. Blood serum, 25°, 30 days.
   Growth: Restricted, gray colored.
   Aerial mycelium: None.
   Soluble pigment: None.
   Liquefaction: None.

6. Egg-media, 25°, 30 days.
   Growth: Spreading, cream-colored, with pinkish tinge.
   Aerial mycelium: Cottony, white tufts all over surface, with pinkish tinge
   showing through.
   Soluble pigment: Pinkish tinge.

7. Potato plug.
   Growth: Wrinkled, cream-colored, becoming yellowish with age (30 days).
   Aerial mycelium: White patches.
   Color of plug: Purplish in 18 days.
8. Carrot, 25°, 22 days.
   Growth: Restricted, later spreading, much raised, folded brownish colored.
   Aerial mycelium: White, powdery, with shade of pink; center dark.
   Color of plug: Dark brown.
9. Starch plate, 25°, 12 days.
   Growth: Cream-colored, round colonies, with faint greenish tinge.
   Aerial mycelium: White, granular in patches over surface.
   Soluble pigment: None.
   Enzymatic zone: 11-13 mm. wide.
10. Gelatin, 18°, 30-35 days.
    Growth: Abundant, dense gray with pinkish tinge, chiefly on surface of liquefied
       portion.
    Aerial mycelium: Abundant, white, powdery on surface of growth.
    Soluble pigment: None.
    Liquefaction: Slow, (0.5 cm. in 35 days) in presence of starch, more rapid in its
       absence.
11. Synthetic solution.
    Growth: Greenish-brown colonies on surface with solid ring in contact with
       glass of tube.
    Aerial mycelium: Usually none or scant white.
    Soluble pigment: Greenish-brown to red brown.
12. Milk, 37°.
    Growth (25°): Yellowish surface zone.
    Coagulation: Begins in 7-8 days, advances very slowly and is not completed
       in 50 days.
    Change of reaction: Faintly (1) to distinctly alkaline (2).
13. Glucose broth, 25°, 12 days.
    Growth: Abundant, cream-colored growth on surface.
    Aerial mycelium: Thin, powdery, rare, white, in tufts.
    Soluble pigment: Dark brown, dissolving downward; often none, particularly if
       growth is limited.

III. BIOCHEMICAL FEATURES.
   1. *Nitrite formation:* Only with starch as source of carbon, not with saccharose or
      glycerin.
   2. *Proteolytic action:* Good in milk; excellent on gelatin, but only good in the
      presence of a 1 per cent starch.
   3. *Change of reaction:* Faintly alkaline in alkaline glucose broth; alkaline in solutions
      containing NaNO$_3$ as source of nitrogen and different sources of carbon;
      distinctly alkaline in acid gelatin, both in presence and absence of starch
      (from P$_H$ 6.2 changed to 7.6); distinctly alkaline in milk.
   4. *Inversion of sugar:* Positive.
   5. *Diastatic action:* Very good; all starch reduced in a 1 per cent solution in 14 days;
      good on plate.
   6. *Growth on cellulose:* None.
   Hab. Isolated from California and Hawaiian soils (161).

Actinomyces exfoliatus Waksman and Curtis

I. MORPHOLOGY.
   1. Spirals.
      Usually none or only a very faint wavy effect of the mycelium (synthetic a on some media, such as cellulose agar and dextrose agar, there is a dis tendency to form spirals.
   2. Conidia.
      Synthetic agar: Oval, 1.0 to 1.5 x 1.2 to 1.8 μ.

II. CULTURAL CHARACTERISTICS.
   1. Synthetic agar.
      Growth: Colorless, later becoming brown with smooth glossy surface veloping deep into the medium; surface growth has at first a tenden crack and peel off, but on successive cultivation this property is lost.
      Aerial mycelium: White patches, soon covering all surface.
      Soluble pigment: Brown, increasing on successive cultivation.
   2. Calcium malate-glycerin agar, 15 days.
      Growth: Restricted, cream-colored surface growth, penetrating to a extent into medium; surface dry, edge myceloid.
      Aerial mycelium: Very thin, powdery, white.
      Soluble pigment: None.
   3. Glucose agar, 15 days.
      Growth: Folded, spreading both on surface and into medium, elevated in c wrinkled, cream-colored with brownish tinge, becoming in 30 da} brown.
      Aerial mycelium: None at first, white patches appear in 30 days.
      Soluble pigment: None.
   4. Nutrient agar.
      Growth: Clear, developing deep into medium, none on surface.
      Aerial mycelium: None.
      Soluble pigment: None.
   5. Blood serum, 37°.
      Growth: Glossy, restricted, cream-colored smear.
      Aerial mycelium: None.
      Soluble pigment: None.
      Liquefaction: None.
   6. Egg-media, 37°.
      Growth: Minute yellowish colonies (5 days), remaining unchanged on tinued incubation.
      Aerial mycelium: None.
      Soluble pigment: None.
   7. Starch plate, 15 days.
      Growth: Restricted, gray colored, changing to brown.
      Aerial mycelium: Light buff-gray.
      Enzymatic zone: Fair, 6–8 mm. wide, incompletely cleared.
   8. Potato plug.
      Growth: Good, somewhat wrinkled, at first gray, later brown colored.
      Aerial mycelium: None.
      Color of plug: Unchanged.
   9. Carrot, 25°, 22 days.
      Growth: Thin, cream-colored, restricted, net-like.
      Aerial mycelium: None.
      Color of plug: Unchanged.

10. Gelatin, 18°.

Growth: Cream-colored, flaky on bottom of liquefied pit.
Aerial mycelium: White or none.
Soluble pigment: None.
Liquefaction: Faint to fair.

11. Synthetic solution.

Growth: Minute colonies through medium.
Aerial mycelium: White, often none at all.
Soluble pigment: Yellow to light brown.

12. Milk, 37°.

Growth (28°): Cream-colored ring on surface.
Coagulation: Usually none, often a soft clot is produced in 8–12 days.
Peptonization: Slow, not clearing as perfectly as in the case of hydrolysis.
Hydrolysis: When the milk does not clot, it hydrolyzes very rapidly and is completed in 8–10 days.
Change of reaction: Strongly alkaline (4).

13. Glucose broth, 25°, 12 days.

Growth: Round, colorless, small colonies on bottom of tube.
Aerial mycelium: None.
Soluble pigment: None.

14. Utilization of different carbon compounds.

Arabinose	4	*Dextrose*	4	*Lactose*	4
Glycerin	3	*Saccharose*	3–5	*Malate*	3
Cellulose	1–3	*Mannite*	3	*Starch*	4
Organic acids	1–2 (tartrate)				

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good with many sources of carbon, often (saccharose and glycerin) only traces are produced.
2. *Proteolytic action:* Fair in milk; faint on gelatin, both in presence and absence of starch.
3. *Change of reaction:* Distinctly alkaline, with different sources of carbon with NaNO$_3$ as source of nitrogen; slightly acid in acid gelatin, changing from P$_H$ 6.2 to P$_H$ 5.8.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Very good, 1 per cent starch all used up in 14 days; fair on plate.
6. *Growth on cellulose:* Good in solution and on plate; clear zone was formed on plate.

Hab. California upland and adobe soil.

Actinomyces flavus Krainsky 1914, p. 685, emend. Waksman and Curtis

1. MORPHOLOGY.

1. Spirals.
   Usually none; very coarse straight branching hyphae on synthetic media; some open spirals may be produced.
2. Conidia.
   Conidia are produced by beading effect and also hyphae breaking up into oval-shaped spores.

II. CULTURAL CHARACTERISTICS.
    1. Synthetic agar.
        Growth: Colonies round, yellow to sulfur-yellow.
        Aerial mycelium: Straw-yellow.
        Soluble pigment: None.
    2. Calcium malate-glycerin agar.
        Growth: Spreading both on and below surface, edge myceloid; color between
          straw yellow and amber yellow (Rdg. XVI, 21').
        Aerial mycelium: Thin, white, powdery over center of growth, leaving wide
          margin uncovered, appears in 3–4 days.
        Soluble pigment: None.
    3. Glucose agar.
        Growth: Restricted, chiefly on surface of medium, center raised and folded;
          sulfur-yellow with brown shade in raised center.
        Aerial mycelium: None at first, scant white to grayish later.
        Soluble pigment: None.
    4. Nutrient agar.
        Growth: Gray, folded, spreading.
        Aerial mycelium: None.
        Soluble pigment: Faint brown.
    5. Blood serum, 25°, 20 days.
        Growth: Spreading, cream-colored.
        Aerial mycelium: Scant, white, in patches.
        Soluble pigment: Brown.
        Liquefaction: None.
    6. Egg-media, 25°, 20 days.
        Growth: Abundant, much wrinkled, cream-colored, later becoming yellow.
        Aerial mycelium: At first traces of white patches which do not develop further.
        Soluble pigment: Purplish, developing only in 7 days.
    7. Starch plate, 25°, 12 days.
        Growth: Spreading, cream-colored, with pink tinge.
        Aerial mycelium: White tufts, with shade of pink.
        Soluble pigment: None.
        Enzymatic zone: 10–11 mm. wide.
    8. Potato plug.
        Growth: Elevated, much wrinkled (barnacle-like), greenish-olive (character-
          istic).
        Aerial mycelium: Thin, white.
        Color of plug: Dark zone around growth.
    9. Carrot, 25°, 22 days.
        Growth: Abundant, raised, restricted, yellowish.
        Aerial mycelium: Thin, sulfur-yellow layer.
        Color of plug: Unchanged.
    10. Gelatin, 18°, 35 days.
        Growth: Heavy, colorless pellicle on bottom of liquefied portion; small yel-
          lowish masses on surface (in contact with glass).
        Aerial mycelium: None.
        Soluble pigment: Dull-brown only in liquefied portion.
        Liquefaction: Rapid (3 cm. in 35 days).
    11. Synthetic solution.
        Growth: Yellowish, round, flaky colonies throughout the medium.
        Aerial mycelium: White to straw-yellow.
        Soluble pigment: None.

12. Milk, 37°. Dark brown soluble pigment.
    Coagulation: 5–6 days.
    Peptonization: Complete in 10–15 days.
    Change of reaction: Distinctly alkaline (4).
13. Glucose broth, 25°, 12 days.
    Growth: Small, white, radiating round colonies on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Mere traces of nitrites are accumulated, with most carbon compounds.
2. *Proteolytic action:* Good on gelatin and milk.
3. *Change of reaction:* Unchanged in gelatin (faintly acid in presence of starch); very acid on glucose broth (Pн changed from 7.9 to 5.2 in 15 days); distinct alkalinity in milk.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Fair to good; zone on plate 10–11 mm. wide in 12 days at 25°.
6. *Growth in cellulose:* Fair, with paper in solution as the only source of carbon.
Hab. Received from Dr. C. B. Lipman, who isolated it from the forming soil of Tortugas Island; isolated also from upland and adobe California soils.

Actinomyces 128

I. MORPHOLOGY.

1. Spirals.
    Usually none on all the media studied. Under the microscope, the growth is found to consist of a large mass of minute tufts; the hyphae are coarse, straight, short, relatively unbranched, beaded; open spirals may be produced in certain instances.
2. Conidia.
    Synthetic agar: Spherical, oval to rod-shaped, 0.75 to 1.0 x 1.0 to 1.5 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
    Growth: Spreading deep into the substratum, yellowish with greenish tinge.
    Aerial mycelium: Powdery, covering all surface with distinct zone formation; Hathi gray (Rdg. LII, 38'''''-b) color.
    Soluble pigment: Greenish yellow.
2. Calcium malate-glycerin agar.
    Growth: Spreading deep into the medium; surface limited only to aerial mycelium; color of growth primuline yellow (Rdg. XVI, 19').
    Aerial mycelium: Powdery, covering center of growth leaving wide uncovered margin; pale mouse gray (Rdg. LI, 15''''') mixed with pale buff.
    Soluble pigment: None.
3. Glucose agar.
    Growth: Restricted, developing only to a very small extent into the medium; edge entire, color yellow with shade of dark, in 30 days turning black.
    Aerial mycelium: Covering all surface, except a narrow margin; color light gull gray (Rdg. LIII, C. G. –9); margin pale buff.
    Soluble pigment: Golden yellow throughout slant.

4. Nutrient agar.

   Growth: Yellowish; the reverse is dark in center, with a yellowish zone and
   outer white zone.

   Aerial mycelium: Abundant, mouse-gray, all over growth.

   Soluble pigment: None.

5. Blood agar, 37°.

   Growth: Green, spreading.

   Aerial mycelium: White.

   Soluble pigment: None.

   Hemolysis: Excellent.

6. Blood serum, 37°.

   Growth: Thin, spreading, gray smear in 4 days, with glossy surface.

   Aerial mycelium: White, developing at an early date (4 days).

   Soluble pigment: None.

   Liquefaction: Rapid in 7–8 days.

7. Egg-media, 37°.

   Growth: Round, yellow colonies.

   Aerial mycelium: Abundant, white, all over growth.

   Soluble pigment: None.

8. Starch plate, 25°, 15 days.

   Growth: Greenish-yellow, spreading growth, developing deep into the medium.

   Aerial mycelium: Gray with tinge of yellow.

   Enzymatic zone: Good (12–15 mm. wide).

9. Potato plug.

   Growth: Sulfur-yellow (4 days), wrinkled.

   Aerial mycelium: Abundant ash-gray, all over growth, appears only in 6–7
   days.

   Color of plug: Narrow black zone around growth, non-spreading.

10. Carrot, 25°, 22 days.

    Growth: Abundant, entire, spreading, yellowish with greenish tinge.

    Aerial mycelium: Membranous, all over surface of growth; white, with yellow
    shade.

    Color of plug: Unchanged.

11. Gelatin, 18°.

    Growth: Yellowish-green surface pellicle consisting of a mass of small colonies;
    there is also a mass of flakes on bottom of liquefied portion.

    Aerial mycelium: Abundant, white, cottony.

    Soluble pigment: None.

12. Synthetic solution, 25°, 15 days.

    Growth: Small, white colonies throughout medium (attached to glass of tube)
    and on surface.

    Aerial mycelium: Ash-gray.

    Soluble pigment: Greenish-yellow.

13. Milk, 37°.

    Growth (25°): Cream-colored to brownish surface ring.

    Coagulation: 3–6 days.

    Peptonisation: Begins soon after coagulation, advances rapidly and is all
    completed in 15–30 days.

    Change of reaction: Faintly alkaline (1).

14. Glucose broth, 25°, 12 days.

    Growth: Thick, sulfur-yellow mass on surface, chiefly in contact with glass of
    tube.

    Aerial mycelium: Cottony, white.

    Soluble pigment: None.

15. Utilization of different carbon-compounds.

Arabinose	0	*Dextrose*	2	*Lactose*	2
Glycerin	1	*Saccharose*	1	*Maltose*	3
Cellulose	1–2			*Starch*	3
Organic acids	1–2				

BIOCHEMICAL FEATURES.
1. *Nitrite formation:* Usually none or mere traces (maltose and glycerin).
2. *Proteolytic action:* Very good on milk; good on gelatin, both in presence and absence of starch.
3. *Change of reaction:* No change, slight acidity or alkalinity with $NaNO_3$, depending on source of carbon; faintly alkaline in milk; distinctly alkaline in glucose broth and in gelatin, both in presence and absence of starch.
4. *Inversion of sugar:* None.
5. *Diastatic action:* Fair, starch not used up in a 1 per cent solution in 20 days; good on plate, zone 12–15 mm. wide.
6. *Growth on cellulose:* None or very scant.
Hab. Oregon adobe soil (128).

Actinomyces fradii Waksman and Curtis

MORPHOLOGY.
1. Spirals.
   None observed on most media; only straight, branching hyphae; on glycerin synthetic agar some spiral formation is observed which is of a dextrorose type.
2. Conidia.
   Synthetic agar: Rod to oval-shaped, 0.5 x 0.75 to 1.25 μ.

CULTURAL CHARACTERISTICS.
1. Synthetic agar.
   Growth: Smooth, spreading, colorless, developing deep into the medium; often a pale yellow orange (Rdg. III, 15–f) shade in reverse. ·
   Aerial mycelium: Thick cottony mass soon covering all surface, of a sea-shell pink color (Rdg. XIV, 11–f).
   Soluble pigment: None.
2. Calcium malate-glycerin agar, 15 days.
   Growth: Spreading, orange-colored, growing deep into medium.
   Aerial mycelium: Cottony patches at first, in 25 days, mycelium covers all surface; color of mycelium is the same as on No. 1, but with lighter colored edge.
   Soluble pigment: None.
3. Glucose agar.
   Growth: Restricted glossy growth on surface of medium; surface of growth forming a net-work in places; edge lichnoid; color cartridge buff (Rdg. XXX, 19″–f).
   Aerial mycelium: None in 15 days, in 25 days patches of characteristic sea-shell pink color appears.
   Soluble pigment: None.
4. Nutrient agar.
   Growth: Yellowish, turning later (10 days) to orange-yellow, restricted.
   Aerial mycelium: None.
   Soluble pigment: None.

5. Blood agar, 37°.
   Growth: Good, brown, crumpled growth is obtained in 2 days, later (21 days)
   changing to reddish colored.
   Aerial mycelium: None.
   Soluble pigment: None.
   Hemolysis: None.
6. Blood serum, 37°.
   Growth: Orange-colored, restricted.
   Aerial mycelium: None.
   Soluble pigment: None.
   Liquefaction: None.
7. Egg-media, 37°.
   Growth: Restricted, wrinkled, orange-colored growth.
   Aerial mycelium: White patches.
   Soluble pigment: None.
8. Starch plate.
   Growth: Spreading, colorless.
   Aerial mycelium: Of the characteristic sea-shell pink color.
   Enzymatic zone: Broad, 12–15 mm.
9. Potato plug.
   Growth: Restricted, orange-colored, characteristic.
   Aerial mycelium: Thin, cream-colored patches.
   Color of plug: Unchanged to faint brown.
10. Carrot, 25°, 22 days.
    Growth: Orange-colored to brownish-orange, spreading, much wrinkled with
    lichnoid margin.
    Aerial mycelium: None.
    Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
    Growth: Cream-colored to brownish dense growth on surface of liquefied
    portion.
    Aerial mycelium: White or none.
    Soluble pigment: None.
    Liquefaction: Rapid, on continued cultivation it becomes slower.
12. Synthetic solution.
    Growth: Minute colonies through medium and on surface.
    Aerial mycelium: None.
    Soluble pigment: None.
13. Milk, 37°.
    Growth (25°): Faint, cream-colored surface ring.
    Coagulation: 10–12 days.
    Peptonization: Begins as soon as coagulation is complete, proceeds rapidly
    and coagulum is all digested in 20 days.
    Hydrolysis: In certain cases, no coagulum is formed, but milk is slowly
    hydrolyzed downward; clearing is not completed in 30 days.
    Change of reaction: Fairly alkaline (2).
14. Glucose broth, 25°, 12 days.
    Growth: Dense, narrow, orange-colored ring on surface in contact with glass;
    also abundant, colorless flaky mass on bottom.
    Aerial mycelium: None.
    Soluble pigment: None.
15. Utilization of different carbon compounds.

5. Blood serum.
   Growth: None.
6. Egg-media, 37°, 7 days.
   Growth: Spreading, cream-colored.
   Aerial mycelium: White cottony tufts all over surface of growth.
   Soluble pigment: None.
7. Starch plate, 25°, 15 days.
   Growth: Grayish brown, with dark ring.
   Aerial mycelium: Gray.
   Ensymatic zone: Fair (8-10 mm. wide), starch not perfectly cleared.
8. Potato plug.
   Growth: Cream-colored at first (4 days), later becoming black (15 days)
   spreading rapidly and surrounding all the plug. The growth of this organism
   and of *A.* 206 results in the destruction of the whole plug in 30 days.
   Aerial mycelium: At first (4–5 days), thin white, later (15 days) becoming
   abundant, white colored with greenish tinge.
   Color of plug: Brown.
9. Carrot, 25°, 22 days.
   Growth: Abundant, enveloping all the surface of the plug.
   Aerial mycelium: Abundant, ash-gray to purplish gray, powdery, all over
   surface.
   Color of plug: Dark brown.
10. Gelatin, 18°, 35 days.
    Growth: Spreading, yellowish, dropping in the form of flakes to bottom of
    liquefied portion.
    Aerial mycelium: White, all over growth.
    Soluble pigment: Faint yellowish in liquefied portion.
    Liquefaction: Slow at first, then rapid, with nearly all tube-liquefied in 35
    days.
11. Synthetic solution.
    Growth: Scant flaky growth on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.
12. Milk, 37°.
    Growth (25°): Abundant surface pellicle, pinkish colored with gray aerial
    mycelium.
    Coagulation: 10–12 days.
    Peptonisation: Begins soon after coagulation is complete, advances with a
    fair speed and in 30 days the clot is all digested.
    Change of reaction: Fairly alkaline.
13. Glucose broth, 25°, 12 days.
    Growth: Thick brown colored, wide ring on surface, chiefly in contact with
    glass.
    Aerial mycelium: Abundant, ash-gray, cottony.
    Soluble pigment: Faint brown or none.

III. BIOCHEMICAL FEATURES.
1. *Nitrite formation:* Fair, with glycerin as a source of carbon.
2. *Proteolytic action:* Good on milk; very good on gelatin, but only good, in the
   presence of 1 per cent starch.
3. *Change of reaction:* Distinctly alkaline in milk and gelatin, both in presence and
   absence of starch; alkaline glucose broth is often turned acid.

4. *Inversion of sugar:* Negative.

5. *Diastatic action:* Fair in plate, saccharogenic power much weaker than amylolytic.

6. *Growth on cellulose:* None or scant.

Hab. A very common soil organism (96).

Actinomyces griseus

This organism was isolated numerous times from the soil.

The term *A. griseus* was used before by Krainsky (22, p. 682), so that the description of the latter is itself an emendation. Although this organism was originally (44) identified with the organism described by Krainsky, under the same name (from description only, without any actual comparison of cultures), this identification should be, therefore, corrected. The culture described here possesses a very strong proteolytic power, while Krainsky stated that his culture was not strong proteolytically.

I. MORPHOLOGY.

1. Spirals.

None on most media; on certain others, such as cellulose agar, spirals are readily formed. Drechsler (13) observed the proliferations of fertile branches at moderately close intervals along the axial hyphae, but no spirals were formed; he is perfectly right in the first, but not exactly in the second, since few closed spirals were formed by this organism on certain media.

2. Conidia.

Synthetic agar: Rod-shaped to short cylindrical, 0.8 x 0.8 to 1.2 μ (0.8 x 0.8–1.5 μ).

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Thin, spreading, developing deep into the medium, at first colorless, then turning olive-buff (Rdg. XL, 2'''–d). This pigment may not be produced on successive transfers.

Aerial mycelium: Thick, powdery mass of a water-green color (Rdg. XLI, 25'''–d).

Soluble pigment: None; reverse of growth brownish with age (24 days).

2. Calcium malate-glycerin agar.

Growth: Growth thin, spreading, penetrating deep into medium; color greenish yellow with dark shade.

Aerial mycelium: Thin, covering all colony except a narrow edge; color approaching tea-green (Rdg. XLVII, 25''''–C).

Soluble pigment: None.

3. Glucose agar.

Growth: Elevated somewhat at center, radiating towards periphery; edge broken; cream-colored, with shade of orange.

Aerial mycelium: Appearing only in dried-up portion of growth in a fine powdery form; white colored, later turning cream-colored.

Soluble pigment: None.

4. Nutrient agar.
   Growth: Abundant, cream-colored to almost transparent.
   Aerial mycelium: Abundant (4 days); color characteristic (as on No. 1).
   Soluble pigment: None.

5. Blood agar, 37°.
   Growth: Extensive, greenish, wrinkled growth is obtained in 24 hours
   Aerial mycelium: White with shade of the characteristic greenish color.
   Soluble pigment: None, often faint brown.
   Hemolysis: Excellent, zone 1 cm. wide in 4 days.

6. Blood serum, 37°.
   Growth: Thin spreading, grayish colored, with glossy surface.
   Aerial mycelium: None.
   Soluble pigment: None.
   Liquefaction: Rapid, begins in 2-3 days.

7. Egg-media, 37°.
   Growth: Spreading, wrinkled cream-colored growth, with greenish tinge.
   Aerial mycelium: None.
   Soluble pigment: None.

8. Starch plate, 15 days.
   Growth: Thin, spreading, transparent.
   Aerial mycelium: Gray.
   Enzymatic zone: 12-15 mm.

9. Potato plug.
   Growth: Much wrinkled, yellowish.
   Aerial mycelium: Powdery, characteristically colored (as on No. 1), all over growth.
   Color of plug: Brownish in upper portion.

10. Carrot.
    Growth: Sulfur-yellow to dirty yellow, folded, spreading.
    Aerial mycelium: Greenish-yellow, all over growth.
    Color of plug: Unchanged.

11. Gelatin, 18°.
    Growth: Greenish-yellow or cream-colored with brownish tinge, developing deep into the substratum.
    Aerial mycelium: White-gray, with greenish tinge.
    Soluble pigment: None.
    Liquefaction: Rapid (3 cm. in 35 days).

12. Synthetic solution.
    Growth: Flakes throughout medium.
    Aerial mycelium: None.
    Soluble pigment: None.

13. Milk, 37°.
    Growth 25° : Cream-colored ring on surface.
    Coagulation: Rapid (2-4 days) clot formation.
    Peptonization: Rapid (3-4 days), clearing up all the milk.
    Enzymolysis: Sometimes the tube is rapidly hydrolyzed, without previous coagulation, which is due to the strong proteolytic action of the organism.
    Change in reaction: Most alkaline (4).

14. Glucose broth, 25°, 12 days.
    Growth: Abundant mass over entire surface of liquid, of yellowish color with greenish tinge; much folded.
    Aerial mycelium: Powdery, of the characteristic sea-green color.
    Soluble pigment: Very faint brown begins to appear in upper portion of liquid

15. Utilization of different carbon compounds.

Arabinose........	3	Dextrose.........	4	Lactose..........	3
Glycerin..........	1	Saccharose.......	1-2	Maltose.........	4
Cellulose..........	0-1	Mannite.........	3	Starch...........	4
Organic acids......	1-3 (malate).				

This is one of the very few species which use glycerin only to a very limited extent.

16. Utilization of different nitrogen compounds.

Ammonium sulfate...............	1-2	Ammonium carbonate........	0
Sodium nitrite..................	1	Acetamide.................	1
Sodium nitrate..................	1-2	Leucin....................	3
Glycocoll.......................	3-4	Casein....................	5
Asparagin......................	3	Fibrin.............	4
Egg-albumin...................	4	Urea.....................	1
Peptone.......................	5		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, in presence of starch, little or none in presence of saccharose or glycerin.
2. *Proteolytic action:* The most active organism in the whole series on milk, gelatin and different proteins.
3. *Change of reaction:* Distinct alkalinity for all sources of carbon used, with NaNO₃ as source of nitrogen; strongly alkaline in milk, glucose broth and gelatin, both in absence and presence of starch.
4. *Inversion of sugar:* None.
5. *Diastatic action:* Very good; 1 per cent starch disappears at the end of 14 days; good on plate.
6. *Growth on cellulose:* Very scant to fair; good growth on sterile soil.

Hab. Isolated from Texas loam, Oregon and California adobe soils.

Actinomyces 218

An organism closely related to the culture previously described (*A. griseus*), but producing a brown pigment on protein containing media and not so strong proteolytically.

I. MORPHOLOGY.

1. Spirals.
   None on most media; often a few short, open spirals are produced.
2. Conidia.
   Oval-shaped, produced abundantly.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
   Growth: Spreading, developing deep into the medium; cream-colored.
   Aerial mycelium: Appears early and covers all surface; powdery, olive-buff to water-green color.
   Soluble pigment: None.

2. Calcium malate-glycerin agar.

    Growth: Thin, spreading, cream-colored, penetrating to some extent into medium; edge erose.

    Aerial mycelium: Thin, netted layer all over growth, surface netted; of a water-green color (Rdg. XLI, 25'''-d).

    Soluble pigment: None.

3. Glucose agar.

    Growth: Restricted, limited to surface, edge entire; yellowish-brown.

    Aerial mycelium: In patches all over growth; pale olive-buff (Rdg. XL, 21''''-f); covering all surface in 30 days.

    Soluble pigment: None

4. Nutrient agar.

    Growth: Glistening, cream-colored at first, later (24 days) becoming brown.

    Aerial mycelium: Abundant, white, all over surface.

    Soluble pigment: Brown.

5. Blood serum, 37°, 15 days.

    Growth: Glossy, elevated, gray colonies.

    Aerial mycelium: Abundant, cream-colored, often in tufts.

    Soluble pigment: Spreading, brown, becoming dark with age.

    Liquefaction: Slow, begins in 25 days.

6. Egg-media, 37°, 15 days.

    Growth: Abundant, cream-colored, spreading, with brownish tinge.

    Aerial mycelium: Abundant, characteristically (olive buff) colored.

    Soluble pigment: Spreading purple.

7. Starch plate, 25°, 12 days.

    Growth: Spreading, cream-colored, with yellowish tinge.

    Aerial mycelium: White powder all over surface of growth.

    Enzymatic zone: 10–12 mm. wide.

8. Potato plug.

    Growth: Brownish

    Aerial mycelium: White, turning olive-buff.

    Color of plug: Unchanged at first, later (20 days) turning faintly brown.

10. Carrot, 25°, 22 days.

    Growth: At first (7 days) very scant, later it develops into a spreading gray growth, with an entire edge and smooth surface.

    Aerial mycelium: White with shade of tea-green.

    Color of plug: Unchanged.

11. Gelatin, 18°, 30 days.

    Growth: Deep growing, cream-colored turning brown, spreading, abundant.

    Aerial mycelium: White in upper portion of growth.

    Soluble pigment: Deep brown, spreading through liquefied portion.

    Liquefaction: Slow in absence of carbohydrate; in presence of starch more rapid (1½ cm. in 35 days).

12. Synthetic solution, 15 days.

    Growth: Scant, white surface pellicle.

    Aerial mycelium: None.

    Soluble pigment: None.

13. Milk, 37°.

    Growth (25°): Brownish ring on surface; soluble brownish pigment.

    Coagulation: None.

    Hydrolysis: Rapid in 10–12 days.

    Change of reaction: Fairly alkaline (2).

15. Glucose broth, 25°, 12 days.

>   *Growth:* Wide, yellowish ring on surface in contact with glass, flaky mass on bottom.
>
>   *Aerial mycelium:* Characteristic yellowish, on surface of upper portion of growth.
>
>   *Soluble pigment:* Faint brown.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with most sources of carbon.
2. *Proteolytic action:* Very good on gelatin, both in presence and absence of starch.
3. *Change of reaction:* Distinctly alkaline in acid gelatin both in presence and absence of starch, from P$_H$ 6.2 to P$_H$ 8.0 and 8.2.
4. *Inversion of sugar:* None.
5. *Diastatic action:* Good on plate.
6. *Growth on cellulose:* Fair to good.

Hab. Isolated from sewage of trickling filter (Plainfield, N. J.).

Actinomyces halstedii Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

>   *Synthetic agar:* Closed spirals born as branches of the hyphae, 7 to 10 μ in diameter. When the organism looses, on continued cultivation, its ability to produce the typical aerial mycelium, no spirals are found in microscopic studies.

2. Conidia.

>   *Synthetic agar:* Oval to rod-shaped, 1.0 to 1.2 x 1.2 to 1.8 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

>   *Growth:* Abundant, heavy, spreading, penetrating into the medium; surface smooth, much raised; color at first light, turning to dark and almost black.
>
>   *Aerial mycelium:* White, turning gull-gray (Rdg. LIII, C. G.) on successive transfers, the culture looses the property of producing aerial mycelium.
>
>   *Soluble pigment:* None; white milky crust on agar around growth.

2. Calcium malate-glycerin agar.

>   *Growth:* Thin, spreading growth penetrating deep into medium; edge entire glossy; color dark with transparent margin.
>
>   *Aerial mycelium:* Thin, covering only center of growth, leaving wide bare margin; aerial mycelium deep mouse gray (Rdg. 15″″′-1).
>
>   *Soluble pigment:* None.

3. Glucose agar.

>   *Growth:* Spreading, extensive, lichnoid; center much elevated, edge wrinkled; colorless at first, with brown center, later becoming all dark brown.
>
>   *Aerial mycelium:* None.
>
>   *Soluble pigment:* None.

4. Nutrient agar.

>   *Growth:* Restricted, wrinkled, cream-colored.
>
>   *Aerial mycelium:* None.
>
>   *Soluble pigment:* None.

5. Blood serum, 37°. No growth.
6. Egg-media, 37°.   No growth, or restricted, round cream-colored patches.
7. Starch plate.
    Growth: Abundant, brownish colored, surface glossy.
    Aerial mycelium: None.
    Ensymatic zone: Very broad (20 mm. and more).   .
8. Potato plug.
    Growth: Abundant, wrinkled with moist surface, cream-colored with green
        tinge.
    Aerial mycelium: White at first, later property lost.
    Color of plug: At first turned black; on continued cultivation property lost,
        plug remaining unchanged.
9. Carrot, 25°, 22 days.
    Growth: Abundant, spreading, raised, surface smooth, moist; color at first
        gray, later turning greenish to dark green.
    Aerial mycelium: None.
    Color of plug: Unchanged.
10. Gelatin, 18°C.
    Growth: Small, cream-colored round masses, dropping to bottom of liquefied
        portion.
    Aerial mycelium: None.
    Soluble pigment: None.
    Liquefaction: Rapid (2–3 cm. in 35 days).
11. Synthetic solution.
    Growth: Small colonies through medium.
    Aerial mycelium: None.
    Soluble pigment: None.
    When glycerin is substituted for saccharose, there is formed an abundant
        flaky growth on bottom of tube.
12. Milk, 37°.
    Growth: Cream-colored surface ring accompanied by rapid hydrolysis at 25°.
    Coagulation: 10 days.
    Peptonization: Slow, begins in 10 days, not completed in 50 days.
    Change of reaction: Fairly alkaline (2).
13. Glucose broth, 25°, 12 days.
    Growth: Small, round, colorless colonies on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

    1. *Nitrite formation:* Good with starch as source of carbon, not with saccharose or
        glycerin.
    2. *Proteolytic action:* Scant on milk, fair on gelatin and glucose broth.
    3. *Change of reaction:* Fair alkalinity in milk; unchanged or slightly acid (in presence
        of 1 per cent starch) in acid gelatin; faintly acid in alkaline glucose broth.
    4. *Inversion of sugar:* Positive.
    5. *Diastatic action:* Excellent; 1 per cent starch in solution disappeared in 7 days;
        zone on plate very good (4).
    6. *Growth on cellulose:* None.
    Hab. New Jersey garden, orchard and meadow subsoils.

Actinomyces hominis Bostroem (4)

I. MORPHOLOGY.
  1. Spirals.
       None on the media studied; aerial mycelium consists only of straight branching hyphae; a few dextrorose spirals are observed when the saccharose of the medium is replaced by glycerin.

II. CULTURAL CHARACTERISTICS.
  1. Synthetic agar, 25°, 15 days.
       Growth: Thin, spreading deep into the medium; color white with shade of yellow; when culture gets older (24 days), the drying up portions turn brown.
       Aerial mycelium: Appears late (15 days); white with olive tinge.
       Soluble pigment: None.
  2. Calcium malate-glycerin agar.
       Growth: Spreeading, yellowish, chiefly on surface of medium.
       Aerial mycelium: Gray, with olive-green tinge, all over growth.
       Soluble pigment: None.
  3. Glucose agar.
       Growth: Yellowish, spreading, chiefly on surface of medium; surface of growth is a fine net-work.
       Aerial mycelium: None in 15 days, later thin, white in patches.
       Soluble pigment: None.
  4. Nutrient agar.
       Growth: Yellowish.
       Aerial mycelium: White.
       Soluble pigment: None.
  5. Blood agar, 37°.
       Growth: Good, rapid growth develops in 24 hours.
       Aerial mycelium: None.
       Soluble pigment: None.
       Hemolysis: Distinct in 4 days, 3–4 mm. zone in 15 days.
  6. Blood serum, 37°.
       Growth: Spreading, transparent, glossy growth.
       Aerial mycelium: None.
       Soluble pigment: None.
       Liquefaction: Rapid (4–8 days).
  7. Egg-media, 37°.
       Growth: Spreading, much wrinkled, yellowish.
       Aerial mycelium: None.
       Soluble pigment: None.
  8. Starch plate, 25°, 15 days.
       Growth: Thin, spreading, transparent.
       Aerial mycelium: None.
       Ensymatic zone: Good, 12–16 mm. wide.
  9. Potato plug.
       Growth: Abundant, wrinkled, yellowish to orange (4 days), later becoming brown (8 days).
       Color of plugs: Unchanged at first, later (17 days) turning brown.
       Aerial mycelium: White patches (4 days), later thin white all over growth (8 days).

10. Carrot, 25°, 22 days.

> *Growth:* At first (7 days), restricted, tawny-olive (Rdg. XXIX, 17″-i) later
> spreading, abundant, wrinkled.
> *Aerial mycelium:* White, with olive-green shade.
> *Color of plug:* Unchanged.

11. Gelatin, 18°, 30 days.

> *Growth:* Abundant, cream-colored, spreading, chiefly on surface, with some
> flakes on bottom of liquefied portion.
> *Aerial mycelium:* None.
> *Soluble pigment:* None.
> *Liquefaction:* Rapid in the absence of starch, very slow in the presence of
> starch.

12. Synthetic solution.

> *Growth:* None. When glycerin is substituted for saccharose there is formed a
> heavy yellowish pellicle on surface of liquid, with a white aerial mycelium.

13. Milk, 37°.

> *Growth* (25°): Abundant, cream-colored surface growth.
> *Coagulation:* 5–6 days; much slower at 25°.
> *Peptonisation:* Begins in 5–6 days, proceeds rapidly and is completed in 20 days.
> *Change of reaction:* Distinctly alkaline (3).

14. Glucose broth, 25°, 12 days.

> *Growth:* Wide, thick, orange-colored ring on surface, in contact with glass of
> tube.
> *Aerial mycelium:* Yellowish, thin on upper portion of ring.
> *Soluble pigment:* None.

III. BIOCHEMICAL FEATURES.

> 1. *Nitrite formation:* Variable, depending on the source of carbon; good to very
>   good with lactose, glycerin and maltose; none with saccharose and cellulose
>   and faint with salts of organic acids.
> 2. *Proteolytic action:* Very good on milk and gelatin, much less on the latter in pres-
>   ence of starch; good on glucose broth.
> 3. *Change of reaction:* Strongly alkaline, with $NaNO_3$ as a source of nitrogen and
>   with different carbohydrates as sources of carbon, particularly is that noticed
>   in the case of maltose, where the P_H increased by 1.4 in 15 days. Distinctly
>   alkaline in acid gelatin and milk, very acid in alkaline glucose broth.
> 4. *Inversion of sugar:* Negative.
> 5. *Diastatic action:* Good on plate.
> 6. *Growth on cellulose:* A good growth was obtained on sterile soil, very scant growth
>   on filter paper.
> *Hab.* Received from Dr. K. F. Meyer; received from Foulerton, who isolated it
>   from abscess of palm of hand in 1911.

Actinomyces lavendulae Waksman and Curtis

I. MORPHOLOGY.

> The aerial mycelium is very coarse, branching.
> 1. Spirals.
>> *Synthetic agar:* Close spirals, 5 to 8μ in diameter, coiled up to form fists.
>> *Dextrose agar:* Spirals formed readily on this as well as on other media; spirals
>> and straight branches break up readily into spores.
> 2. Conidia.
>> *Synthetic agar:* Abundant, oval, 1.0 to 1.2 x 1.6 to 2.0μ.

II. **Cultural Characteristics.**

  1. Synthetic agar.

Growth: Thin, spreading, colorless, developing deep into the medium.

Aerial mycelium: Cottony in spots, at first white, later turning deep vinaceous-lavender (Rdg. XLIV, 65'''–d); on successive transfer, the aerial mycelium may not be produced at all or remains white.

Soluble pigment: None.

  2. Calcium malate-glycerin agar.

Growth: Minute cream-colored colonies on surface.

Aerial mycelium: None in 15 days; in 25 days lavender colored white patches appear all over surface.

Soluble pigment: None.

  3. Glucose agar.

Growth: Thick, yellowish, spreading growth, penetrating to some extent into the medium, edge myceloid.

Aerial mycelium: Thick, cottony, all over surface, white with lavender tinge.

Soluble pigment: None.

  4. Nutrient agar.

Growth: Wrinkled, gray.

Aerial mycelium: None.

Soluble pigment: Brown.

  5. Blood serum, 37°.

Growth: Small, gray colonies.

Aerial mycelium: None.

Soluble pigment: Brown, spreading.

Liquefaction: None.

  6. Egg-media, 37°.

Growth: A fine cream-colored net-work on surface of medium.

Aerial mycelium: None.

Soluble pigment: None.

  7. Starch plate, 25°, 15 days.

Growth: Non-spreading, glistening, transparent, developing deep into the medium.

Aerial mycelium: Lavender colored.

Enzymatic zone: Good (3).

  8. Potato plug.

Growth: Thin, wrinkled smear, cream-colored to yellowish.

Aerial mycelium: None.

Color of plug: Turns black; on continued cultivation property of coloring plug may be nearly lost.

  9. Carrot.

Growth: Abundant, raised, wrinkled, spreading, brownish colored.

Aerial mycelium: Powdery over most of surface of growth; white, with faint shade of lavender.

Color of plug: Unchanged.

  10. Gelatin, 18°.

Growth: Creamy to brownish, restricted on surface, with granule-like growth on bottom of liquefied portion.

Aerial mycelium: None to small white patches in contact with wall of tube.

Soluble pigment: Brown or none at all.

Liquefaction: Slow.

11. Synthetic solution.

Growth: Small, colorless, radiating colonies attached to glass; mass of floati colonies on surface.

Aerial mycelium: Characteristic lavender; property lost on transfer.

Soluble pigment: None.

12. Milk, 37°. Soluble brown pigment.

Growth (25°): Cream-colored ring.

Coagulation: None.

Hydrolysis: 20–30 days; at 25° hydrolysis proceeds more rapidly (4–5 days) proceeding from surface downwards.

Change of reaction: Strongly alkaline (4).

13. Glucose broth, 25°, 12 days.

Growth: Abundant, flaky mass on bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Positive, with starch as source of carbon, negative with saccharose and glycerin.

2. *Proteolytic action:* Fair on milk, faint to fair on gelatin.

3. *Change of reaction:* Acid on gelatin, both in presence and absence of starch, from P_H 6.2 to P_H 5.6 and 5.8; alkaline in milk.

4. *Inversion of sugar:* Positive, often a negative reaction is obtained.

5. *Diastatic action:* Very good; starch disappeared in 14 days in a 1 per cent starch solution; very good on plate, enzymatic zone 12–15 mm. in 15 days.

6. *Growth on cellulose:* Scant although definite.

Hab. Isolated from New Jersey orchard, California and Oregon white land; also isolated by Drechsler (13).

Actinomyces lipmanii Waksman and Curtis

This organism, or rather group, is closely related to the *A. diastaticus* of the writer and that described by Krainsky, and *A. rutgersensis*, particularly in some cultural and biochemical characters (strong diastatic action); but it differs from the other two organisms in morphology and certain cultural characters, as can be readily seen from the following description.

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: None on the media studied; hyphae straight, branching, showing in places some curvature.

2. Conidia.

Synthetic agar: 0.8 to 1.1 x 1.0 to 1.5 μ.

I CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, much raised, at first colorless, wrinkled, later becoming light brown.

Aerial mycelium: White, turning gray (Rdg. LIII, 6) with white margin and white patches.

Soluble pigment: None.

2. Calcium malate-glycerin agar, 25°, 12 days.

> *Growth:* Small, hyaline at first, later becoming dark, spreading as a very thin layer on surface and to a limited extent into the medium.
>
> *Aerial mycelium:* Appears early, covering all the growth; mouse-gray, with wide white margin.
>
> *Soluble pigment:* None.

3. Glucose agar.

> *Growth:* Light yellow, irregular, spreading growth, developing deep into the medium.
>
> *Aerial mycelium:* None in 20 days.
>
> *Soluble pigment:* None.

4. Nutrient agar.

> *Growth:* Yellowish, glossy, radially wrinkled.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None; when glycerin is present a characteristic green color is produced.

5. Blood agar, 37°.

> *Growth:* Much folded, abundant, of a characteristic dirty gray color, with shade of green.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.
>
> *Hemolysis:* None.

6. Blood serum, 37°.

> *Growth:* Cream-colored to almost transparent, thin, spreading.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.
>
> *Liquefaction:* Medium (12–15 days).

7. Starch plate, 25°, 15 days.

> *Growth:* Transparent at first, later becoming dark.
>
> *Aerial mycelium:* None.
>
> *Enzymatic zone:* Good to very good (3).

8. Potato plug.

> *Growth:* Abundant, wrinkled growth, cream-colored.
>
> *Aerial mycelium:* White patches at first (4 days), later becoming gray and covering all surface.
>
> *Color of plug:* Purplish.

9. Carrot, 25°, 22 days.

> *Growth:* Abundant, spreading, folded, of a dirty-gray color.
>
> *Aerial mycelium:* At first scant, later ash-gray covering all surface.
>
> *Color of plug:* Unchanged.

10. Gelatin.

> *Growth:* Cream-colored flaky growth falling to bottom of liquefied portion.
>
> *Aerial mycelium:* White-gray.
>
> *Soluble pigment:* None.
>
> *Liquefaction:* Rapid to medium.

11. Synthetic solution.

> *Growth:* Flakes throughout medium, settling to bottom of tube.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.

12. Milk, 37°.

Growth (25°): Cream-colored ring on surface.

Coagulation: 8–9 days; the clot is often at (25°) nothing more than a thickening of the milk.

Peptonisation: Begins soon after all the milk is coagulated, proceeds with a fair speed and is completed in 20–30 days.

Change of reaction: Fairly alkaline (2).

13. Glucose broth, 25°, 12 days.

Growth: White ring on surface; abundant colorless flaky mass on bottom of tube.

Aerial mycelium: None.

Soluble pigment: None.

14. Utilization of different carbon compounds.

Arabinose.......... 0	*Dextrose*.......... 3	*Lactose*........... 3
Glycerin........... 3	*Saccharose*......... 3	*Starch*............. 3
Cellulose.......... 0	*Mannite*.......... 3	

Addition of glycerin to organic or inorganic solid media results in the production of a distinctly characteristic, abundant green-colored growth.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good, produced with different sources of carbon.

2. *Proteolytic action:* Good in milk and on gelatin.

3. *Change of reaction:* Increase in alkalinity with different sources of carbon and NaNO₃ as a source of nitrogen.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Very good; all starch used up in 14 days in 1 per cent starch solution; very good on plate, zone 12–15 mm. wide in 15 days.

6. *Growth on cellulose:* None, or very scant.

Hab. This is one of the most common groups of soil actinomycetes. Isolated from the New Jersey garden and orchard, Iowa, Louisiana, California, North Dakota, Hawaiian, Alaska, Texas and Oregon adobe soils.

Actinomyces 168

I. MORPHOLOGY.

1. Spirals.

Numerous, closed or open, broad spirals on all media.

2. Conidia.

Oval shaped to elliptical.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Spreading deep into the medium, light sulfur yellow, later turning cadmium yellow (Rdg. III, 17); on repeated transfers for 3 years, yellow color is lost, color of growth is nearly white.

Aerial mycelium: Appears early (2–3 days), thin, white, with ash-gray patches.

Soluble pigment: Empire yellow (Rdg. IV, 21); property lost in 2 years.

2. Calcium malate-glycerin agar.

Growth: Colorless, later becoming cream-colored, penetrating deep into the medium; surface growth limited to aerial mycelium; edge myceloid.

Aerial mycelium: Thin layer all over growth; mouse-gray with white margin.

Soluble pigment: None.

3. Glucose agar.

> Growth: Restricted, both on surface and into the medium, surface much folded, raised.
>
> Aerial mycelium: Thin, powdery, white layer over upper portion of slant (in tube); in 30 days it covers all growth, pale gray color with white margin.
>
> Soluble pigment: Yellowish green.

4. Nutrient agar.

> Growth: Glistening, wrinkled, white.
>
> Aerial mycelium: Abundant, white all over growth.
>
> Soluble pigment: None.

5. Blood agar, 37°.

> Growth: Brownish.
>
> Aerial mcyelium: White.
>
> Soluble pigment: None.
>
> Hemolysis: Narrow zone.

6. Blood serum.

> Growth: Thin, brownish smear in 4 days; often growth is restricted, compact, orange-colored.
>
> Aerial mycelium: None at first, later (15 days) white over edge of growth.
>
> Soluble pigment: None.
>
> Liquefaction: Slow, begins in 15 days.

7. Egg-media, 25°.

> Growth: Thin, spreading brownish growth, radially wrinkled (6 days) remaining unchanged.
>
> Aerial mycelium: None.
>
> Soluble pigment: None.

8. Potato plug.

> Growth: Abundant, wrinkled, cream-colored in 7 days.
>
> Aerial mycelium: White, all over growth (4 days), later (30 days) becoming powdery.
>
> Color of plug: Unchanged at first, later (30 days) turning faint brown.

10. Starch plate.

> Growth: White spreading.
>
> Aerial mycelium: Light gray.
>
> Enzymatic zone: Broad (15–18 mm. wide), not all the starch is perfectly cleared.

11. Gelatin, 18°, 30 days.

> Growth: Abundant, yellowish, spreading pellicle.
>
> Aerial mycelium: Abundant, white.
>
> Soluble pigment: Coloration of gelatin golden to faint brown.
>
> Liquefaction: Rapid (3 cm. in 35 days, in presence of starch, 1½–2 cm. in absence of starch) to medium.

12. Synthetic solution.

> Growth: Heavy yellowish pellicle on surface.
>
> Aerial mycelium: White, with gray tinge.
>
> Soluble pigment: Yellow to deep yellow.

13. Milk, 37°.

> Growth (25°): Sulfur-yellow surface ring, with yellow soluble pigment.
>
> Coagulation: 5–8 days.
>
> Peptonization: Begins in 6–8 days, advances rapidly and is completed in about 20 days.
>
> Hydrolysis: May take place in certain cultures in place of coagulation.
>
> Change of reaction: Faintly alkaline (1).

14. Glucose broth, 25°, 12 days.
    Growth: Thin, yellow pellicle, the part immersed in the liquid having spongy
    appearance.
    Aerial mycelium: Thin, white.
    Soluble pigment: Golden.
15. Effect of different carbon compounds.

Arabinose 0	*Dextrose* 5	*Lactose* 3–4
Saccharose 2	*Maltose* 4	*Cellulose* 1–3
Mannite 5	*Starch* 3	*Organic acids* 1–3

III. BIOCHEMICAL FEATURES.
   1. *Nitrite formation:* Excellent, with different sources of carbon.
   2. *Proteolytic action:* Fair in milk; fair to good on gelatin, both in presence and
      absence of starch.
   3. *Change of reaction:* None or little change with small amount of growth; with heavy
      growth, reaction becomes distinctly alkaline with $NaNO_3$ as source of nitrogen
      and different sources of carbon; strongly alkaline in acid gelatin, both in pres-
      ence and absence of starch (P_H changed from 6.2 to 8.0); faintly alkaline in
      alkaline glucose broth and milk.
   4. *Inversion of sugar:* None.
   5. *Diastatic action:* Excellent, all starch disappeared in a 1 per cent solution in 14
      days; good on plate.
   6. *Growth on cellulose:* Scant to fair with all methods studied.
   Hab. California fertilized soil (168).

Actinomyces madurae (Vincent (39)) Lehmann and Newmann

I. MORPHOLOGY.
   1. Spirals.
      Usually none on most of the media studied; only straight, branching hyphae
      are obtained; often a few open or closed spirals are found.

II. CULTURAL CHARACTERISTICS.
   1. Synthetic agar.
      Growth: Cream-colored, spreading, chiefly below the surface of the medium.
      Aerial mycelium: Thin, powdery, white, appearing early (4 days).
      Soluble pigment: None.
   2. Calcium malate-glycerin agar.
      Growth: Spreading, cream-colored, developing deep into the medium.
      Aerial mycelium: Gray, all over growth.
      Soluble pigment: None.
   3. Glucose agar.
      Growth: Thin, glistening, pinkish, spreading on surface and into the medium.
      Aerial mycelium: None in 15 days, later white powdery zone over margin of
      growth.
      Soluble pigment: None.
   4. Nutrient agar.
      Growth: Abundant, cream-colored, spreading growth.
      Aerial mycelium: White, appears late (15–20 days).
      Soluble pigment: None.

5. Blood agar, 37°, 15 days.
   Growth: Dark brown, spreading, rapid growth, obtained in 2 days.
   Aerial mycelium: None.
   Soluble pigment: None.
   Hemolysis: Sharply defined, transparent zone 2–4 mm. in width.
6. Blood serum, 37°.
   Growth: Glossy, round, yellowish colonies obtained in 4 days.
   Aerial mycelium: Thin, white in 4 days.
   Soluble pigment: None.
   Liquefaction: Rapid (4–8 days).
7. Egg-media, 37°, 15 days.
   Growth: Thin, spreading, wrinkled yellowish growth.
   Aerial mycelium: Thin white patches.
   Soluble pigment: None.
8. Starch plate, 25°, 15 days.
   Growth: Thin, spreading transparent.
   Aerial mycelium: None.
   Ensymatic zone: Very good, 12–16 mm. wide.
9. Potato plug, 25°.
   Growth: Abundant, wrinkled, sinking into the plug, yellow colored.
   Aerial mycelium: None at first, later (15 days) white to gray all over growth.
   Color of plug: Unchanged at first, later (17 days) turning faint brown.
10. Carrot, 25°, 22 days.
    Growth: Scant, transparent, yellowish, later spreading.
    Aerial mycelium: None or white-grayish patches.
    Color of plug: Unchanged.
11. Gelatin, 18°, 30 days.
    Growth: At first cream-colored, turning to greenish flaky masses, dropping to
    bottom of liquefied portion; brownish in exposed portion.
    Aerial mycelium: Scant white patches.
    Soluble pigment: None.
    Liquefaction: Rapid (2–3 cm. depth of liquefied gelatin in tube in 35 days).
12. Synthetic solution.
    Growth: None. When glycerin is substituted for saccharose, a few colorless
    flakes are formed on bottom of tube.
13. Milk, 37°.
    Growth (25°): Cream-colored ring; action on milk proteins slow at 25°.
    Coagulation: 3–4 days at 37°, while at 25° only thickening is observed followed
    by slow digestion.
    Peptonization: Begins in 3–4 days, proceeds rapidly and is all completed in
    10–30 days. Rapidity of coagulation and peptonization is very variable,
    depending on mother culture, amount of inoculum, etc.
    Change of reaction: Faintly (1) to distinctly (3) alkaline.
14. Glucose broth, 25°, 12 days.
    Growth: Abundant, flaky, colorless colonies on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.
15. Utilization of different carbon compounds.

Dextrose	2	*Glycerin*	1	*Saccharose*	1
Cellulose	0	*Organic acids*	1–2		
		(lactate)			

III. BIOCHEMICAL FEATURES

An organism under this name received from Král, was described by Foulerton and Price (see 16) as weakly proteolytic and with no diastatic action. The organism studied here proved to be very strongly proteolytic and possessing good diastatic properties.

1. *Nitrite formation:* Very limited, none (lactate) to fair with other sources of carbon.
2. *Proteolytic action:* Excellent on milk, peptone and gelatin (less in presence of starch).
3. *Change of reaction:* Fairly alkaline with $NaNO_3$ as the only source of nitrogen and different sources of carbon; distinctly alkaline in acid gelatin, in absence of available carbohydrates (only faintly alkaline in presence of starch); faintly alkaline in alkaline glucose broth and milk.
4. *Inversion of sugar:* Negative.
5. *Diastatic action:* Very good on plate.
6. *Growth on cellulose:* Scant.

Hab. The culture was received from Dr. K. F. Meyer of the Hooper Foundation, San Francisco, Cal., who had it from the American Museum of Natural History, received from Parke Davis Co., Feb., 1911, (No. 05); received by them in May, 1902, from Král; also received by the writer, from Parke Davis Co. (No. 01136).
    The description given above was based on the first culture; the second strain made a weaker growth on synthetic media.

Actinomyces pheochromogenus Conn 1917, p. 16

This organism has been isolated by the writer and Curtis from the soil, and has been also isolated and described by Conn (10).

I. MORPHOLOGY.

1. Spirals.
    Dextrose agar: Many spirals of the narrower type, open, elongated; the spirals are sinistrorose.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.
    Growth: The growth is developing deep into the medium, is at first colorless, later becoming brown to almost black.
    Aerial mycelium: Abundant, all over growth, white colored, with brownish shade.
    Soluble pigment: Black.
2. Calcium malate-glycerin agar.
    Growth: Thin, spreading, penetrating to some extent into the medium; edge erose; color yellowish with brown tinge.
    Aerial mycelium: Thin, white, cottony, covering central portion of surface, leaving rather wide, bare margin.
    Soluble pigment: Brown to dark brown.
3. Glucose agar.
    Growth: Restricted, much folded, growing deep into medium; color brown to dark brown.
    Aerial mycelium: White, covering only portions of surface, later spreading over all surface; in 35 days, dark color of growth makes it look quite dark.
    Soluble pigment: Brown.

4. Nutrient agar.

   Growth: Thin, cream-colored, turning gray.

   Aerial mycelium: None.

   Soluble pigment: Deep brown.

5. Blood agar, 15 days, 37°C.

   Growth: Brown.

   Aerial mycelium: None.

   Soluble pigment: None.

   Hemolysis: None.

6. Blood serum, 37°.

   Growth: Spreading, brown, developing late.

   Aerial mycelium: None.

   Soluble pigment: Chocolate brown.

   Liquefaction: Faint.

7. Egg-media, 37°.

   Growth: Slow, dark brown.

   Aerial mycelium: White, net-like all over surface of growth.

   Soluble pigment: Purplish, turning dark brown.

8. Starch plate, 12 days, 25°.

   Growth: Spreading, brownish becoming later dark brown.

   Aerial mycelium: White cottony tufts scattered over surface.

   Soluble pigment: None.

   Enzymatic zone: 6–8 mm. wide.

9. Potato plug.

   Growth: Brown to almost black, wrinkled growth in 4–5 days.

   Aerial mycelium: White patches.

   Color of plug: Dark to almost black zone around growth.

10. Carrot, 25°, 22 days.

    Growth: Scant, dark brown, late, developing only after 15 days.

    Aerial mycelium: Scant, white.

    Color of plug: Black soluble pigment is spreading over plug even before any appreciable growth is obtained.

11. Gelatin, 18°.

    Growth: Abundant surface growth, spreading, cream-colored, turning brown in exposed portions.

    Aerial mycelium: None at first, later white aerial mycelium may be found.

    Soluble pigment: Deep brown.

    Liquefaction: Slow to medium.

12. Synthetic solution (glycerin in place of saccharose).

    Growth: Brownish heavy pellicle on surface with a few flakes throughout the medium.

    Aerial mycelium: Abundant, buff colored.

    Soluble pigment: Brown, spreading.

13. Milk, 37°.   Soluble brown pigment at the end of 30 days.

    Growth (25°): Dark to almost black surface ring; soluble black pigment.

    Coagulation: Late (15–20 days); the soft coagulum settles to the bottom.

    Peptonization: Slow, whey clouded.

    Hydrolysis: The milk may hydrolyze without previous coagulation, particularly at 25°.

    Change of reaction: Faintly alkaline (1).

14. Glucose broth, 12 days, 25°.

>*Growth:* Dense, wrinkled, wide ring on surface of liquid only in contact with glass.
>
>*Aerial mycelium:* Thin, white.
>
>*Soluble pigment:* Deep brown.

III. BIOCHEMICAL FEATURES.

' 1. *Nitrite formation:* Very good with glycerin as a source of carbon.

2. *Proteolytic action:* Faint in milk; good on gelatin.

3. *Change of reaction:* Faintly alkaline in milk, often unchanged; distinctly alkaline in acid gelatin (from P_H 6.2 to 7.7 and 8.4); faintly alkaline in glucose broth.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Fair; tested only on plate, zone 6-8 mm. wide in 12 days at 25°.

6. *Growth on cellulose:* Usually scant.

Hab. Isolated from New Jersey orchard soil; also obtained from Dr. H. J. Conn of the New York Agricultural Experiment Station, who described and named this organism.

Actinomyces poolensis Taubenhaus 1918, p. 446

I. MORPHOLOGY.

1. Mycelium.

>A very fine branching mycelium is produced; spirals are usually not produced, only in certain instances, a fine wavy effect is observed or close spirals may be formed.

2. Conidia.

>Oval to elliptical.

II. CULTURAL CHARACTERISTCS.

1. Synthetic agar.

>*Growth:* Thin, colorless, spreading, developing deep into the medium; when saccharose is replaced by dextrose or glycerin, growth becomes very abundant, with yellow on reverse.
>
>*Aerial mycelium:* White with shade of gray appearing late.
>
>*Soluble pigment:* None.

2. Calcium malate—glycerin agar.

>*Growth:* Thin, spreading, cream-colored chiefly below the surface; edge myceloid; surface smooth.
>
>*Aerial mycelium:* Thin over entire surface, leaving narrow margin uncovered; color light mouse-gray (Rdg. LI, 15''''''-b) with creamy edge.
>
>*Soluble pigment:* None.

3. Glucose agar.

>*Growth:* Abundant, light brown, chiefly on surface, but also to some extent below the surface; surface glossy; center raised; edge entire.
>
>*Aerial mycelium:* Usually none; certain strains (214) may produce a cottony aerial mycelium of a pale olive-bluff color (Rdg. XL, 21'''-f).
>
>*Soluble pigment:* None.

4. Nutrient agar.

>*Growth:* Translucent, yellowish growth, the surface of which presents a fine network.
>
>*Aerial mycelium:* None.
>
>*Soluble pigment:* None.

5. Blood agar, 37°, 7 days.
   Growth: Green, often yellowish-green, finely netted.
   Aerial mycelium: None.
   Soluble pigment: None to faint dark.
   Hemolysis: None.
6. Blood serum, 37°, 7 days.
   Growth: Thin, spreading, gray smear.
   Aerial mycelium: None or scant white patches.
   Soluble pigment: None.
   Liquefaction: None or faint, appearing late (25 days).
7. Egg-media, 37°, 7 days.
   Growth: Thin, spreading, wrinkled, brownish colored.
   Aerial mycelium: None.
   Soluble pigment: Faint brown.
8. Starch plate, 25°, 12 days.
   Growth: Restricted, cream-colored.
   Aerial mycelium: White, cottony tufts all over surface.
   Enzymatic zone: 9–10 mm. wide.
9. Potato plug.
   Growth: Thin, reddish-brown, sinking into plug.
   Aerial mycelium: None.
   Color of plug: Purplish, appearing late (17 days).
10. Carrot, 25°, 22 days.
    Growth: Thin, restricted, finely folded, brownish colored smear.
    Aerial mycelium: None.
    Color of plug: Unchanged.
11. Gelatin, 18°, 35 days.
    Growth: Small brownish flakes at bottom of liquefied portion.
    Aerial mycelium: None.
    Soluble pigment: None or faint yellow.
    Liquefaction: Rapid to medium; about 2 cm. of depth of gelatin in tube is lique-
        fied in 35 days.
12. Synthetic solution.   Very scant growth on saccharose; when glycerin is substi-
        tuted into the solution, the results are as follows:
    Growth: Abundant, brownish flaky growth on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: Faint brownish.
13. Milk, 37°.
    Growth (25°, 20 days): Brownish ring on surface.
    Coagulation: 4–5 days.
    Peptonization: Begins in 4–5 days, advances very rapidly and is completed in
        9–10 days.
    Hydrolysis: The milk is often hydrolyzed, without any visible coagulation.
    Change of reaction: Strongly alkaline (4).
14. Glucose broth, 25°, 12 days.
    Growth: Thin, brownish ring on surface, in contact with glass.
    Aerial mycelium: None.
    Soluble pigment: None.
15. Utilization of different carbon compounds.

Arabinose 0	*Dextrose* 1	*Lactose* 2
Glycerin 4	*Saccharose* 1	*Maltose* 1
Cellulose 0–1	*Mannite* 1	*Starch* 3
Organic acids 0–1		

III. BIOCHEMICAL FEATURES.

    1. *Nitrite formation:* None or only traces with certain sources of carbon; fair with
        glycerin.

    2. *Proteolytic action:* Excellent on milk; very good on gelatin; fair on peptone.

    3. *Change of reaction:* None or only faint alkalinity with some sources of carbon and
        with NaNO$_3$ as only source of nitrogen; distinctly alkaline in gelatin and glu-
        cose broth; strongly alkaline in milk.

    4. *Inversion of sugar:* Negative.

    5. *Diastatic action:* Fair, zone on plate 9–10 mm. wide in 12 days, height of tube
        above control 5 mm.

    6. *Growth on cellulose:* None or very scant, with methods used.

    Hab. Isolated by Dr. J. Taubenhaus from diseased sweet potato tubers and by the
        writer several times from the soil; a closely related organism (214) was received
        from Dr. C. B. Lipman, who isolated it from forming soil on Tortugas Island.

Actinomyces purpeochromogenus Waksman and Curtis

I. MORPHOLOGY.

    1. Spirals.
        Synthetic agar: None.
        Starch agar: Few, imperfect spirals observed.

    2. Conidia.
        Spherical spores, 0.75 to 1.0 in diameter.

II. CULTURAL CHARACTERISTICS.

    1. Synthetic agar.
        Growth: Slow, restricted, chiefly on surface of medium, surface smooth, center
        raised, color at first gray, later becoming brown with purplish tinge; margin
        yellow.
        Aerial mycelium: Produced late, brownish purple to black; this is present as a
        thin, dry surface layer over growth, which makes it hard to distinguish it from
        the latter.
        Soluble pigment: Brown to dark brown.

    2. Calcium malate-glycerin agar.
        Growth: Spreading, chiefly on surface, gray colored in the medium, black on sur-
        face of medium, margin spreading.
        Aerial mycelium: None.
        Soluble pigment: None in 15 days.

    3. Glucose agar.
        Growth: Abundant, restricted, developing 2–3 mm. into medium, 1 mm. above
        medium; at first gray, turning brown to dark brown.
        Aerial mycelium: Brown to dark-brown aerial mycelium covering surface of
        growth.
        Soluble pigment: Faint dark, later turning dark brown.

    4. Nutrient agar.
        Growth: Gray to brownish, penetrating into medium; surface growth becomes
        dark brown to almost black.
        Aerial mycelium: None.
        Soluble pigment: Brown.

    5. Blood serum, 37°. No growth.

    6. Egg-media, 37° and 25°. No growth or limited, thin, cream-colored smear.

7. Starch plate, 16 days, 25°.

   Growth: The growth consists of a mass of small, dark-brown, individual colonies.

   Aerial mycelium: Deep purple, with a glossy surface.

   Soluble pigment: None.

   Ensymatic zone: 4–5 mm., reduction incomplete.

8. Potato plug.

   Growth: Restricted orange to orange-red colonies in 3–4 days, turning dark red in 15 days.

   Aerial mycelium: None.

   Color of plug: Unchanged, becoming faintly brown with age.

9. Carrot. No growth.

10. Gelatin, 30 days, 18°.

    Growth: Slow, brownish colored.

    Aerial mycelium: None.

    Soluble pigment: Brown.

    Liquefaction: Slow.

11. Synthetic solution.

    Growth: Scant flakes on bottom of flask.

    Aerial mycelium: None.

    Soluble pigment: None.

12. Milk, 37°.

    Growth (25°): Dark brownish ring on surface, in contact with glass; pinkish flakes in milk; brownish pigment in liquefied portion.

    Coagulation: 10 days.

    Peptonization: Begins in 10–12 days, advances slowly and is not completed in 50 days. In certain cases the digestion may proceed more rapidly and is completed in 30 days.

    Change of reaction: Faintly alkaline (1).

13. Glucose broth.

    Growth: Few flakes on bottom of flask.

    Aerial mycelium: None.

    Soluble pigment: None.

III. Biochemical Features.

   1. *Nitrite formation:* None with different carbon sources.
   2. *Proteolytic action:* Fair in milk and gelatin.
   3. *Change of reaction:* Faintly alkaline in milk.
   4. *Inversion of sugar:* Fair.
   5. *Diastatic action:* Scant; 1 per cent starch is not used in more than 4 weeks; faint diastatic action upon plate.
   6. *Growth on cellulose:* Usually scant.
   Hab. Isolated from California adobe soil.

Actinomyces reticuli Waksman and Curtis

I. Morphology.

   1. Spirals.

      Synthetic agar: None. Whirl formation characteristic of this as well as of the following species; the difference between this and the following organism is that the branches of this species do not form secondary whirls. This species represents a distinct group of organisms, widely separated from the other actinomycetes; several strains have been isolated which are as distinct from the

original *reticuli* as different species can be from one another, but in the same time form with it one group, that distinguishes them from the other species. The characteristic feature of this group of organisms is the peculiar method of branching of the aerial mycelium. Instead of the straight or curled branches usually found in the *Actinomyces* species on this medium, the organisms of this group produce a whirl of branches from a common point; these whirls are formed at intervals on the main hyphae. Conidia have been demonstrated to be formed from these branches.

Dextrose agar: Whirl formation predominant. Also tendency to form spirals, which are of the sinistrorose type.

· 2. Conidia.

Synthetic agar: Spherical, 1.0 to 1.4 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Colorless, with yellowish tinge on surface, later becoming brownish, spreading, chiefly deep in the medium; surface growth limited to aerial mycelium.

Aerial mycelium: Thin, white, cottony, appearing in 7–12 days, forming a fine net-work with large holes in net (about 0.5 mm.).

Soluble pigment: None.

2. Calcium malate-glycerin agar.

Growth: Scanty, colorless, developing to some extent into the medium.

Aerial mycelium: Small round patches of a naphthalene yellow color (Rdg. XVI, 23′–f).

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted, brownish, center much raised above surface, penetrating only to a small extent into the medium.

Aerial mycelium: Cottony, covering all growth, of a naphthalene yellow color.

Soluble pigment: Brownish, diffusing through the medium.

4. Nutrient agar.

Growth: Wrinkled, gray, later becoming brownish.

Aerial mycelium: None.

Soluble pigment: Brown, spreading.

5. Blood serum, 37°.

Growth: Restricted, gray colonies.

Aerial mycelium: None.

Soluble pigment: Brown, spreading.

Liquefaction: None.

6. Egg-media, 37°.

Growth: Thin, spreading, radially wrinkled, gray.

Aerial mycelium: Gray with dark center.

Soluble pigment: Purple zone around growth.

7. Starch plate, 15 days.

Growth: Brownish gray.

Aerial mycelium: Lavender colored.

Ensymatic zone: Narrow, 4–5 mm. wide.

8. Potato plug.

Growth: Medium, gray with black center.

Aerial mycelium: Ash-gray.

Color of plug: Black.

9. Carrot, 25°, 22 days.
   Growth: Abundant, yellowish colored, spreading, surface smooth, dry.
   Aerial mycelium: None.
   Color of plug: Unchanged.
10. Gelatin, 18°, 35 days.
    Growth: Small, flaky, gray turning to brown, sinking to the bottom of the liquefied gelatin.
    Aerial mycelium: Thin, white patches.
    Soluble pigment: Faint to dark brown.
    Liquefaction: Medium (1 cm. in 35 days) to slow.
11. Synthetic solution.
    Growth: Small white flakes on bottom of tube, with abundant gray growth on surface.
    Aerial mycelium: White.
    Soluble pigment: None.
12. Milk, 3°. Soluble brown pigment.
    Growth: Dark ring on surface, with no effect upon milk (only darkening) at 25° in 20 days.
    Coagulation: 4–5 days.
    Peptonization: Begins as soon as coagulation is complete, advances very slowly so that not all the coagulum is digested in 50 days.
    Change of reaction: Unchanged.
13. Glucose broth, 25°, 12 days.
    Growth: Large-sized colonies on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: Brown.
14. Utilization of different carbon compounds.

Dextrose	4	*Saccharose*	1
Cellulose	3	*Maltose*	3
Organic acid (acetate)	1–2		

15. Utilization of different nitrogen compounds.

Ammonium sulfate	0	*Peptone*	4
Sodium nitrite	1	*Ammonium carbonate*	0
Sodium nitrate	1	*Acetamide*	0
Glycocoll	3	*Leucin*	3
Asparagin	3	*Fibrin*	4
Egg-albumin	1	*Urea*	0

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair with different sources of carbon.
2. *Proteolytic action:* Fair in milk, good on gelatin, very good on fibrin, good on peptone, faint on egg-albumin.
3. *Change of reaction:* Unchanged in milk, unchanged or faint acidity with most proteins and amino acids, with glycerin as source of energy; faint acidity in alkaline glucose broth; fairly alkaline in acid gelatin.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Fair, a 1 per cent starch solution not used up in 20 days; no reducing sugars found; faint to fair in plate, zone only 4–5 mm. wide in 15 days.
6. *Growth on cellulose:* Scant.
Hab. Isolated from Iowa, California upland and adobe soils.

Actinomyces reticulus-ruber n. sp.

I. MORPHOLOGY.

    1. Spirals.

        Synthetic agar: None. Whirl formation, both primary and secondary; primary whirls consisting of fewer branches, hyphae thicken at place of whirl formation.

        Dextrose agar: Tendency to form spirals predominant. No spirals on calcium malate agar.

    2. Conidia.

        Synthetic agar: None observed (?).

        Dextrose agar: Oval shaped.

II. CULTURAL CHARACTERISTICS.

    1. Synthetic agar, 15 days.

        Growth: Abundant, spreading, chiefly deep into the medium, where it is colorless; surface growth usually pink, often colorless.

        Aerial mycelium: Thin, rose to pink colored, leaving a wide uncovered margin.

        Soluble pigment: None.

    2. Calcium malate-glycerin agar.

        Growth: Spreading on surface and deep into the medium; edge erose; growth colorless in the medium, red to rose-red on surface.

        Aerial mycelium: Covering nearly all surface in a thin layer, white with rose pink shade.

        Soluble pigment: None.

    3. Glucose agar.

        Growth: Extensive, spreading, penetrating deep into the medium; edge entire; color of surface growth rose-red (Rdg. XII, 71).

        Aerial mycelium: Cottony, covering all surface; color white with shade of pink due to color of growth.

        Soluble pigment: None.

    4. Nutrient agar.

        Growth: At first (4 days) red with yellowish margin, later becoming Acajou red (Rdg. XIII, 1'–i).

        Aerial mycelium: None.

        Soluble pigment: Brown.

    5. Blood serum, 37°.

        Growth: Glossy, grayish colonies appear early (4 days) and remain nearly unchanged.

        Aerial mycelium: None.

        Soluble pigment: Spreading, brown.

        Liquefaction: None.

    6. Egg-media, 37°, 15 days.

        Growth: Spreading, wrinkled, brownish red in color, characteristic.

        Aerial mycelium: Thin, white, with pink shade due to red color of growth.

        Soluble pigment: Rapidly spreading, soluble black pigment, penetrating in 15 days through all the slant.

    7. Starch plate, 25°, 15 days.

        Growth: White with red tinge.

        Aerial mycelium: Lavender colored.

        Enzymatic zone: Fair, 7–8 mm. wide.

8. *Potato plug.*

Growth: At first (4 days) cream-colored, later pink patches appear and finally (15 days) all growth becomes of a dark red color.

Aerial mycelium: At first white, later (14 days) surface is changed to pink, then to red.

9. *Carrot, 25°, 22 days.*

Growth: At first (7 days). restricted, brownish; later the growth is spreading, thin, smooth, of a characteristic red-beet color.

Aerial mycelium: None.

Color of plug: Unchanged.

10. *Gelatin, 18°, 35 days.*

Growth: Yellowish red to dragon pink (Rdg. XII, 6') surface growth; growth also consisting of colorless flakes on bottom of liquefied portion.

Aerial mycelium: Cottony, hemosa pink (Rdg. I, 1) color.

Soluble pigment: Brown.

Liquefaction: Rapid (1½–2 cm. in 35 days).

11. *Synthetic solution.*

Growth: Small, white, cottony colonies all throughout medium.

Aerial mycelium: Faint pink.

Soluble pigment: None to faint brown.

When glycerin is substituted for saccharose the colonies are deep red.

12. *Milk, 37°. Soluble brown pigment.*

Growth (25°): Abundant, red colored surface growth; rose-colored aerial mycelium.

Coagulation: 4–6 days.

Peptonization: Begins soon after coagulation is complete, advances very slowly, so that in 50 days not all the coagulum is digested.

Change of reaction: Unchanged.

13. *Glucose broth, 25°, 12 days.*

Growth: Thick, flaky mass on bottom of tube.

Aerial mycelium: None.

Soluble pigment: Brown to none.

14. *Utilization of different carbon compounds.*

Arabinose	0	*Dextrose*	4	*Lactose*	2
Glycerin	3	*Saccharose*	1	*Mallose*	4
Cellulose	4	*Mannite*	1	*Starch*	5
Organic acids (acetate)	1–2				

III. **B**IOCHEMICAL FEATURES.

1. *Nitrite formation:* Excellent with different sources of carbon.

2. *Proteolytic action:* Fair in milk; fair on gelatin, both in presence and absence of starch; faint on glucose broth.

3. *Change of reaction:* No change of slight alkalinity with NaNO$_3$ as source of nitrogen and different carbon compounds as sources of energy; faintly alkaline in acid gelatin; very distinctly acid in glucose broth.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Scant in solution, fair on plate.

6. *Growth on cellulose:* Very good on paper in solution.

Hab. New Jersey orchard and California upland soils.

Actinomyces roseus Krainsky 1914, p. 682, emend. Waksman and Curtis

I. MORPHOLOGY.
  1. Spirals.
      Numerous open and closed spirals produced on the different synthetic media; type of spirals dextrorose.
  2. Conidia.
      Synthetic agar: Oval, 1.0 to 1.2 x 1.5 to 3.0 μ.

II. CULTURAL CHARACTERISTICS.
  1. Synthetic agar.
      Growth: Thin, spreading, colorless, penetrating deep into the medium.
      Aerial mycelium: Thin, pale brownish vinaceous (Rdg. XXXIX, 5′′′-f); property may be lost on successive transfers, but can easily be regained on transferring to favorable media.
      Soluble pigment: None.
  2. Calcium malate-glycerin agar.
      Growth: Spreading, colorless, growing deep into the medium.
      Aerial mycelium: White to rose colored.
      Soluble pigment: None.
  3. Glucose agar.
      Growth: Extensive, colorless, spreading on and below the surface of the medium, edge entire.
      Aerial mycelium: Covering all surface, except wide margin; flesh-pink color (Rdg. XIII, 5′-f), with white margin.
      Soluble pigment: None.
  4. Nutrient agar.
      Growth: White, later turning yellowish.
      Aerial mycelium: None.
      Soluble pigment: None.
  5. Blood serum, 37°.
      Growth: Thin gray smear, later (15 days) turning brown.
      Aerial mycelium: None.
      Soluble pigment: None, often dark.
      Liquefaction: None.
  6. Egg-media, 37°.
      Growth: Thin, spreading, greenish-yellow.
      Aerial mycelium: None.
      Soluble pigment: Narrow purple zone around growth.
  7. Starch plate, 25°, 12 days.
      Growth: Spreading, colorless.
      Aerial mycelium: White cottony tufts all over surface, with shade of pink.
      Ensymatic zone: 6–7 mm. wide.
  8. Potato plug.
      Growth: Much wrinkled, brownish.
      Aerial mycelium: None.
      Color of plug: Brown; on continued cultivation property lost, color of plug remaining unchanged.
  9. Carrot.
      Growth: Scant, restricted, wrinkled, light brownish.
      Aerial mycelium: None.
      Color of plug: Unchanged.

10. Gelatin, 18°.
   Growth: Small, cream-colored colonies, sinking into the medium.
   Aerial mycelium: None or thin white.
   Soluble pigment: Brown, spreading into the unliquefied portion.
   Liquefaction: Slow.
11. Synthetic solution.
   Growth: Flakes throughout liquid.
   Aerial mycelium: None.
   Soluble pigment: None.
12. Milk, 37°.
   Growth (25°): Brownish ring on surface in contact with glass.
   Coagulation: None.
   Hydrolysis: 10–15 days.
   Change of reaction: Strongly alkaline (4).
13. Glucose broth.
   Growth: Flakes on bottom, creamy ring on surface.
   Aerial mycelium: None.
   Soluble pigment: Brown.
14. Utilization of different carbon compounds.

Arabinose 1	*Dextrose* 3	*Starch* 3
Glycerin 3	*Saccharose* 2	
Cellulose 1	*Mannite* 1	
Organic acids 0–1		

III. BIOCHEMICAL FEATURES.
   1. *Nitrite formation:* Good, in presence of different sources of carbon.
   2. *Proteolytic action:* Faint in milk.
   3. *Change of reaction:* Slight increase in alkalinity with NaNO$_3$ as source of nitrogen and different carbon compounds; strong alkalinity in milk.
   4. *Inversion of sugar:* None.
   5. *Diastatic action:* Very good, all starch used up in 14 days from a 1 per cent solution; on continued cultivation, culture loses somewhat its diastatic power, giving only a fair action on starch, by plate method.
   6. *Growth on cellulose:* None.
   Hab. Isolated from New Jersey garden soil and identified by Waksman and Curtis (1916) as belonging to the above species although comparison with Krainsky's culture, as is the case of the other cultures, was impossible.

Actinomyces ruber Krainsky 1914, p. 686

I. MORPHOLOGY.
   1. Spirals.
      Usually none on all media studied. Mycelium consists of straight branching hyphae, often radiating from a common center. A few spirals may be formed.

II. CULTURAL CHARACTERISTICS.
   1. Synthetic agar.
      Growth: Abundant, spreading, developing deep into the medium; red colored.
      Aerial mycelium: Abundant, cottony, chrom-orange color (Rdg. II, 11).
      Soluble pigment: None.

2. Calcium malate-glycerin agar.

> *Growth:* Spreading, orange colored (Rdg. III, 15) with lighter margin penetrating deep into the medium.
>
> *Aerial mycelium:* Cottony, cadmium yellow (Rdg. III, 17), developing at an early date.
>
> *Soluble pigment:* None.

3. Glucose agar.

> *Growth:* Restricted, abundant, chiefly on surface, margin entire, coral red (Rdg. XIII, 5′) color, with yellowish growth in the medium.
>
> *Aerial mycelium:* Powdery, thin, white, with pink tinge, leaving uncovered margin.
>
> *Soluble pigment:* None.

4. Nutrient agar.

> *Growth:* Restricted, elevated, wrinkled surface growth, olive-green (Rdg. XXXI, 25″) color.
>
> *Aerial mycelium:* Thin, ash-gray.
>
> *Soluble pigment:* Brown.

5. Blood agar, 37°.

> *Growth:* Green.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Dark-gray, slowly spreading.
>
> *Hemolysis:* Narrow colorless zone.

6. Blood serum, 37°.

> *Growth:* Yellow, with red center.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Brown, spreading.
>
> *Liquefaction:* None.

7. Egg-media, 37°.

> *Growth:* Spreading, radially wrinkled, brick-red color.
>
> *Aerial mycelium:* None at first, later (15 days) rose-color on edge of growth.
>
> *Soluble pigment:* Faint dark zone.

8. Starch plate, 15 days.

> *Growth:* Abundant, spreading, red.
>
> *Aerial mycelium:* Pinkish-red.
>
> *Ensymatic zone:* Fair, 8–10 mm. wide, not perfectly clear.

9. Potato plug, 25°.

> *Growth:* Elevated, wrinkled, greenish (4 days).
>
> *Aerial mycelium:* Red, with yellow margin.
>
> *Color of plug:* Black zone around growth.

10. Carrot, 25°, 22 days.

> *Growth:* Abundant, spreading, raised, brownish colored.
>
> *Aerial mycelium:* Cottony, light salmon orange (Rdg. II, 11–d), all over surface of growth.
>
> *Color of plug:* Dark brown.

11. Gelatin, 18°, 30 days.

> *Growth:* Yellow flakes.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Brown, spreading.
>
> *Liquefaction:* Medium.

12. Synethic solution.

> *Growth:* Colorless flakes throughout the medium.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.

13. Milk, 37°.
>   Growth (25°, 20 days): Dark surface ring, with tinge of red.
>   Coagulation: 3–4 days.
>   Peptonisation: Begins in 3–4 days, advances very rapidly, and is completed in about 10 days.
>   Change of reaction: Fairly alkaline (2).

14. Glucose broth, 25°, 12 days.
>   Growth: Small spongy colonies on surface of liquid with a red ring in contact with glass.
>   Aerial mycelium: Powdery, white, in upper portion of ring.
>   Soluble pigment: None.

15. Utilization of different carbon compounds. When glycerin is substituted for saccharose, into the synthetic solution, a characteristic growth is produced consisting of colonies throughout the medium having a red center and a wide colorless margin; a pink aerial mycelium is formed on the surface colonies.

Arabinose..... 0–trace	Dextrose......... 3	Lactose........... 3
Glycerin............ 3	Saccharose....... 2–5	Maltose........... 4
Cellulose........... 2	Mannite..........2–3	Starch............ 4
Organic acids....... 0 (oxalate)–2 (acetate).		

16. Utilization of different nitrogen compounds.

Ammonium sulfate........... 2	Ammonium carbonate.......... 1
Sodium nitrite............... 1	Acetamide................... 1–2
Sodium nitrate............... 2	Leucin...................... 3–4
Glycocoll................... 2–5	Peptone.................... 3–4
Asparagin.................. 3–5	Casein..................... 2–3
Egg-albumin................ 2–4	Fibrin.................. 3–5
Urea...................... 3	

III. BIOCHEMICAL FEATURES.

1. Nitrite formation: Faint to fair with most sources of carbon, good with maltose and lactose and excellent with arabinose, although the growth on this source of carbon was only very limited.

2. Proteolytic action: Good on milk, good to very good on peptone, casein, fibrin; faint on egg-albumin.

3. Change of reaction: In most cases distinctly alkaline, with NaNO₃ as source of nitrogen, and with different sources of carbon; with dextrose, mannite and glycerin reaction is unchanged; reaction is unchanged with most nitrogen sources and glycerin as source of carbon, acid with glycocoll and leucin, alkaline with peptone; distinctly acid in alkaline glucose broth (P_H changed from 7.9 to 5.6 in 15 days).

4. Inversion of sugar: Positive.

5. Diastatic action: Fair on plate: height of hydrolyzed starch by tube method, above control, 14–16 mm.

6. Growth on cellulose: Good with all methods; no clear zone formed on plate.

Hab. Received from Dr. C. B. Lipman, who isolated it from the forming soil of Tortugas Island.

Actinomyces rutgersensis Waksman and Curtis

This organism corresponds very closely, on certain media and in some biochemical characters, with *A. diastaticus* described by Krainsky (22). This organism, as well as the *A. diastaticus* and *A. lipmanii*, represent closely related forms (culturally) and are among the most common of the soil actinomycetes.

I. MORPHOLOGY.
   1. Spirals.
      Synthetic agar: Abundant, both close and open type; hyphae fine, long, branching.
   2. Conidia.
      Synthetic agar: Spherical and oval, 1.0 to 1.2 μ in diameter; tendency to bi-polar staining.

II. CULTURAL CHARACTERISTICS.
   1. *Synthetic agar:*
      Growth: Fair, thin, spreading, penetrating deep into the medium, at first colorless, later becoming brownish to almost black, particularly on repeate d transfers.
      Aerial mycelium: At first thin white, later becoming pale dull-gray (Rdg. LIII, C. G.).
      Soluble pigment: None.
   2. Calcium malate-glycerin agar.
      Growth: Spreading, developing deep into the medium, cream-colored; edge myceloid.
      Aerial mycelium: Thin, covering all the growth, leaving narrow edge uncovered; pale dull-gray in color, with white patches.
      Soluble pigment: None.
   3. Glucose agar.
      Growth: Abundant, brown colored on surface, penetrating only very slightly into the medium, edge myceloid; in 30 days color of growth is black, with wide cream-colored margin.
      Aerial mycelium: None in 15 days, white patches in 30 days.
   4. Nutrient agar.
      Growth: Thin, wrinkled, cream-colored.
      Aerial mycelium: None.
      Soluble pigment: None.
   5. Blood serum, 37°.
      Growth: Restricted, glossy, gray smear.
      Aerial mycelium: None.
      Soluble pigment: None.
      Liquefaction: None.
   6. Egg-media, 37°.
      Growth: Thin, gray colored smear.
      Aerial mycelium: Thin, powdery, white.
      Soluble pigment: Purple spreading pigment appears late (30 days).
   7. Starch plate, 25°, 12 days.
      Growth: Gray, spreading.
      Aerial mycelium: Gray.
      Ensymatic zone: Fair to good, 8–10 mm. wide.

8. Potato plug.

   Growth: Abundant, much folded, white-gray colored at first (4 days), later becoming brownish, till finally (30 days) it is all brown.

   Aerial mycelium: White patches appearing late (15 days).

   Color of plug: Unchanged.

9. Carrot, 25°, 22 days.

   Growth: Large, round, restricted, cream-colored colonies; surface smooth, dry, much raised.

   Aerial mycelium: None.

   Color of plug: Unchanged.

10. Gelatin, 18°, 30 days.

    Growth: Cream-colored spreading with flakes dropping to bottom of tube.

    Aerial mycelium: White, thin patches over growth.

    Soluble pigment: None at first; on continued cultivation, a light brown color is produced in liquefied portion only.

    Liquefaction: Medium; all tube liquefied in 30–35 days; in the presence of 1 per cent starch only half of the tube is liquefied in the same period of time.

11. Synthetic solution (no growth with saccharose; glycerin used in this solution).

    Growth: Thin white pellicle; few flakes through medium.

    Aerial mycelium: Thin, white.

    Soluble pigment: None.

12. Milk, 37°.

    Growth (25°): Cream-colored ring on surface.

    Coagulation: 4–6 days.

    Peptonization: Begins as soon as coagulation is complete, advances very slowly, so that in 50 days not all the coagulum is digested.

    Change of reaction: Fairly alkaline (2).

13. Glucose broth, 25°, 12 days.

    Growth: Small colorless colonies on bottom of tube.

    Aerial mycelium: None.

    Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good to very good with different sources of carbon.

2. *Proteolytic action:* Good in milk; very good on gelatin, both in presence and absence of starch.

3. *Change of reaction:* Fair alkalinity in milk; practically unchanged in glucose broth; distinctly alkaline in gelatin, both in presence and absence of starch.

4. *Inversion of sugar:* None.

5. *Diastatic action:* Excellent; all starch used up in 7 days in a 1 per cent solution; fair to good on plate.

6. *Growth on cellulose:* Scant.

Hab. Common soil organism. Isolated from New Jersey garden orchard and timothy soils; Louisiana, California, North Dakota, Alaska, Texas, and Colorado soils.

Actinomycetes scabies (Thaxter) Güssow (Syn. *Streptothrix chromogenus* Gasperini, *Oospora scabies* Thaxter)

I. MORPHOLOGY.

1. Spirals:

   This organism did not form many spirals on the media under the conditions as studied; the aerial myphae are usually long and branched; often few spirals are produced.

Synthetic agar: Wavy or slightly curved; on other media (cellulose) agar spiral formation took place; the spirals are of a dextrorose type.

2. Conidia.

Conidia are produced readily on most synthetic media; these are more or less cylindrical, 0.8–1.0 x 1.2–1.5 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, cream-colored, chiefly on surface of medium, wrinkled, raised.

Aerial mycelium: White, scarce; some strains may not produce any at all.

Soluble pigment: None at first, later a brown pigment may develop.

2. Calcium malate-glycerin agar.

Growth: Good, spreading both on surface and into medium; faint yellowish in color, later turning dark.

Aerial mycelium: Thin layer all over colony, only leaving edge uncovered, mouse-gray with white margin and patches.

Soluble pigment: None.

3. Glucose agar.

Growth: Restricted, folded, cream-colored, edge entire.

Aerial mycelium: None.

Soluble pigment: None; surface agar around medium colored milky white.

4. Nutrient agar.

Growth: Growth consists of round, entire colonies; surface at first smooth, later raised, lichnoid, often becoming wrinkled; color white to straw colored, opalescent to opaque.

Aerial mycelium: Usually absent, often a scant white is produced.

Soluble pigment: Brown, spreading.

5. Blood agar, 37°, 15 days.

Growth: Brownish to gray.

Aerial mycelium: None.

Soluble pigment: Dark brown.

Hemolysis: None.

6. Blood serum, 37°, 7 days.

Growth: Slow, restricted, much folded, yellowish to brown colonies.

Aerial mycelium: None.

Soluble pigment: Brown, forming a zone around growth.

Liquefaction: None.

7. Egg-media, 37°, 7 days.

Growth: Small, wrinkled, brown colonies, later becoming dark brown.

Aerial mycelium: None.

Soluble pigment: Narrow purplish to black zone.

8. Starch plate, 12 days.

Growth: Thin, transparent, spreading.

Aerial mycelium: Scant white.

Enzymatic zone: Questionable diastase production.

9. Potato plug, 7 days.

Growth: Gray, opalescent, later turning jet black, wrinkled colonies covering all the plug.

Aerial mycelium: None; in some strains an ash gray mycelium appears.

Color of plug: Black.

10. Carrot.
    Growth: Scant, thin, yellowish, semitransparent.
    Aerial mycelium: None.
    Color of plug: Unchanged.

11. Gelatin, 18°.
    Growth: Cream-colored, turning brown in portions exposed to the air.
    Aerial mycelium: Scant white in 37 days.
    Soluble pigment: Deep brown.
    Liquefaction: Slow at first, later becoming more rapid so that in 35 days 2.7 cm. of the gelatin is digested.

12. Synthetic solution (glycerin in place of saccharose).
    Growth: An abundant mass of colorless flakes and colonies on bottom of tube.
    Aerial mycelium: None.
    Soluble pigment: None.

13. Milk, 37°.
    Deep brown pigment begins to develop in 7 days in the form of a surface ring, and in 30 days the whole tube turns brown.
    Growth: Brown colored ring with greenish tinge at 25°.
    Coagulation: 5–10 days; certain strains many not show any coagulation at all, but a slow hydrolysis.
    Peptonization: Begins soon after coagulation is complete (5–10 days), proceeds at fair speed, and is completed in 15–30 days.
    Change of reaction: Fairly alkaline (2).

14. Glucose broth.
    Growth: Growth on the surface in the form of a ring, consisting of many small colonies, which soon fuse together; this may settle to the bottom.
    Aerial mycelium: None.
    Soluble pigment: Brown, dissolving downward.

15. Utilization of different carbon compounds.

Arabinose 4	*Dextrose* 3	*Lactose* 4
Glycerin 3	*Saccharose* 2–3	*Starch* 3
Cellulose 1–2	*Mannite* 3	
Organic acids 1		

16. Utilization of different nitrogen compounds.

Ammonium sulfate .... 0 (T)	*Ammonium carbonate* .... 0 (T)
Sodium nitrite 1–3	*Acetamide* 1–2
Sodium nitrate 1–2	*Leucin* 2–3
Glycocoll 2–3	*Peptone* 3–4
	(reaction alkaline, ammonia produced late, after 20 days).
Asparagin 2	*Fibrin* 3–5
	(distinct ammonia accumulation).
Egg-albumin 2–3	*Casein* 3–4
Urea 1–2	

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* None to mere traces with nearly all sources of carbon, with the exception of glycerin when a fair nitrite formation took place; often none is found even with glycerin.

2. *Proteolytic action:* Fair on milk, weak on peptone, casein, fibrin, and egg-albumin, although a sufficient amount of the material is split to produce a very good growth; fair on gelatin.

3. *Change of reaction:* With NaNO$_3$ as a source of nitrogen, the medium becomes alkaline with nearly all sources of carbon studied; distinctly alkaline in acid gelatin (P$_H$ 6.2 changed to 7.9 in 35 days at 18°); alkaline with peptone, casein, fibrin, glycocoll, etc., faint acidity with leucin and acetamide (glycerin as source of energy); distinctly alkaline in milk; unchanged in alkaline glucose broth.

4. *Inversion of sugar:* Positive.

5. *Diastatic action:* Questionable on plate, hydrolysis only to erythro-reaction; same on tube.

6. *Production of tyrosinase:* Very good. Although most organisms made a good growth on tyrosin agar (with tyrosin as the only source of nitrogen), only some strains of *A. scabies*, and *A. chromogenus* 205 produced a soluble dark brown pigment, spreading readily through the medium. This would tend to indicate that these two organisms are the only ones that are able to produce a true tyrosinase, or, if, as Beijerinck (3) suggested, tyrosinase is a mixture of two enzymes, only these two organisms of the whole group are able to produce both enzymes.

7. *Growth on cellulose:* Fair with certain methods (plate and reprecipitated cellulose in solution); none on paper in solution; clear zone is not formed on plate.

Hab. Isolated from potato scab, also from the soil.

A number of cultures received from other investigators were compared with the strain described above, and many of them showed distinctive differences, particularly as to the production of a brown soluble pigment on organic media and aerial mycelium on synthetic media. Some strains attacked the carrot and potato plug very readily, so that within 2 weeks the plug was all shrivelled up and covered with ash-gray powdery aerial mycelium; one strain made no growth on carrot and only a scant smear was produced on potato; several other strains fell between the two extremes. The weakly growing strains which made a scant or poor growth on potato and carrot, without pigmenting the plug dark brown, produced also no dark pigment on tyrosin agar and attacked the milk proteins only slowly.

Actinomyces verne Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: No spirals; the branching of the hyphae is so close as to produce the impression of making whirls.

Starch agar: Few short coiled side branches.

2. Conidia.

Synthetic agar: None demonstrated.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

Growth: Abundant, spreading, wrinkled, much elevated above surface, also developing into medium, margin lichnoid, surface glossy; color yellowish at first, later developing a brown tinge.

Aerial mycelium: No true aerial mycelium demonstrated.

Soluble pigment: Elm-green (Rdg. XVII, 27–Km) diffusing through medium; on repeated transfer, the culture produces, instead of a green pigment, a deep brown soluble pigment.

2. Calcium malate-glycerin agar.

> *Growth:* Thin, restricted, wrinkled, chiefly on surface, color avellaneous (Rdg. XL, 17′′′–b).
>
> *Aerial mycelium:* None in 20 days, later scant white.
>
> *Soluble pigment:* Faint brown.

3. Glucose agar.

> *Growth:* Abundant, much folded, chiefly on surface of medium; center raised, edge entire; color gray with purplish-brown tinge.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Faint, brown.

4. Nutrient agar.

> *Growth:* Small grayish colonies, with depression in center, later becoming wrinkled.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.

5. Blood serum, 37°.

> *Growth:* Restricted, cream-colored.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.
>
> *Liquefaction:* Medium (10–12 days).

6. Egg-media, 37°.

> *Growth:* Thin, much wrinkled, cream-colored.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.

7. Starch plate, 25°, 16 days.

> *Growth:* Brownish colored, scant, restricted growth.
>
> *Aerial mycelium:* None to a faint brown pellicle on surface of growth.
>
> *Enzymatic zone:* 8–10 mm. wide.

8. Potato plug.

> *Growth:* Wrinkled, cream-colored (4 days), later (15 days) becoming gray.
>
> *Aerial mycelium:* None, sometimes scanty white mycelium is produced.
>
> *Color of plug:* Becomes faint brown with age of culture (30 days).

9. Carrot, 25°, 22 days.

> *Growth:* Spreading, raised, much folded, brownish.
>
> *Aerial mycelium:* None.
>
> *Color of plug:* Unchanged.

10. Gelatin, 18°, 30 days.

> *Growth:* Small cream-colored colonies.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* Green, property lost on continued cultivation.
>
> *Liquefaction:* Rapid to medium; liquefied portion in tube 1.5 cm. deep in 30 days.

11. Synthetic solution.

> *Growth:* Small colonies on bottom of tube.
>
> *Aerial mycelium:* None.
>
> *Soluble pigment:* None.
>
> When glycerin is substituted for saccharose, there is formed a mass of brownish flakes on bottom of tube, with a faint brown soluble pigment.

12. Milk, 37°.

> *Growth* (25°): Pinkish-brown ring on surface.
>
> *Coagulation:* 4–5 days.
>
> *Peptonization:* Begins in 5 days, advances rapidly and completed in 18–25 days.
>
> *Change of reaction:* Fairly alkaline (2).

13. Glucose broth.

   Growth: Small flakes on bottom of tube.
   Aerial mycelium: None.
   Soluble pigment: Faint brown to none.

14. Utilization of different carbon compounds.

Arabinose......... 0	*Dextrose*......... 1	*Lactose*............ 2
Glycerin.......... 1	*Saccharose*....... 1–2	*Maltose*........... 2
Cellulose.......... 1–3	*Mannite*......... 3	*Starch*............. 1

15. Utilization of different nitrogen compounds (glycerin as a source of energy).

Ammonium sulfate............ 0	*Ammonium carbonate*......... 0
Sodium nitrite................ 1	*Acetamide*.................... 1
Sodium nitrate............... 1	*Leucin*....................... 3
Glycocoll.................... 3	*Casein*...................... 2–3
Asparagin.................. 2–3	*Fibrin*....................... 2
Egg-albumin................. 2	*Urea*........................ 1
Peptone.................... 3	

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Good with different sources of carbon.
2. *Proteolytic action:* Very good in milk and on the different pure proteins (fibrin, casein, egg-albumin).
3. *Change of reaction:* No change, slight acidity or slight alkalinity with NaNO₃, as source of nitrogen, depending on the source of carbon; unchanged or faint alkalinity with different proteins and amino acids, except with leucin, where the change of reaction is towards acidity.
4. *Inversion of sugar:* Positive.
5. *Diastatic action:* Very good, starch reduced chiefly to erythrodextrin; all starch disappeared in 14 days in a 1 per cent solution. Diastatic power somewhat deteriorated on continued cultivation, giving only a good reaction (3).
6. *Growth on cellulose:* Usually good, when cellulose, either in the form of paper or dissolved and reprecipitated, is the only source of carbon, but none on plate.

Hab. Isolated from California upland soil.

Actinomyces violaceus-caesari Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.
   Synthetic agar: Numerous open spirals.
   Calcium-malate agar: Broad spirals; both spirals and straight aerial mycelium break up into spores.
   Dextrose agar: Numerous, open; some have corkscrew effect; spirals dextro-rose.
2. Conidia.
   Calcium-malate agar: Oval-shaped to elongated.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.
   Growth: Colonies glossy, much wrinkled, gray at first, later becoming bluish.
   Aerial mycelium: Appears late, white with no shade of gray.
   Soluble pigment: Plum-purple (Rdg. XXIV, 57–m), turning darker to almost brown with age of culture.

2. Calcium malate-glycerin agar, 15 days.

    Growth: Restricted, developing deep into medium, only aerial mycelium on surface of medium; color of growth is blue.

    Aerial mycelium: White, with purplish tinge, due to pigment of underlying growth.

    Soluble pigment: Faint blue, rapidly spreading.

3. Glucose agar, 15 days.

    Growth: Restricted, on surface, penetrating to some extent into the medium; at first gray, later turning red to almost brick-red.

    Aerial mycelium: White patches over surface of growth.

    Soluble pigment: None.

4. Nutrient agar, 15 days.

    Growth: Thin, cream-colored smear.

    Aerial mycelium: None.

    Soluble pigment: None.

5. Blood serum, 37°.

    Growth: Small, restricted, gray colonies, developing only in 15 days.

    Aerial mycelium: None.

    Soluble pigment: None.

    Liquefaction: None.

6. Egg-media, 37°.

    Growth: Thin, finely wrinkled, of a grayish color (5–6 days), later (15 days) showing a dark center.

    Aerial mycelium: None.

    Soluble pigment: None.

7. Starch plate, 12 days.

    Growth: Individual, round, restricted colonies, of a bluish violet color.

    Aerial mycelium: In the form of tufts, gray in centre and white at the periphery.

    Soluble pigment: None.

    Ensymatic zone: 12–14 mm. wide, but hydrolysis is not complete (reddish tinge on addition of iodine solution); the starch is hydrolyzed chiefly to the erythrodextrin stage.

8. Potato plug.

    Growth: Cream-colored, wrinkled, in 4–5 days; in 15 days color of growth turns yellowish.

    Aerial mycelium. None.

    Color of plug: Unchanged.

9. Carrot: No growth.

10. Gelatin, 18°, 35 days.

    Growth: Small, cream-colored colonies on surface, may drop to bottom of liquefied portion.

    Aerial mycelium: None.

    Soluble pigment: None.

    Liquefaction: Medium or slow.

11. Synthetic solution, 15 days.

    Growth: Small flakes on bottom of tube.

    Aerial mycelium: None.

    Soluble pigment: Blue, property almost lost with age of organism on continued cultivation. On replacing the saccharose by glycerin, the blue color is formed readily.

12. Milk, 37°.

Growth: Gray ring on surface.

Coagulation: 10–12 days; sometimes milk is not clotted.

Peptonization: Slow, complete only in 50 days.

Change of reaction: Faintly alkaline (1).

13. Glucose broth, 12 days.

Growth: Fine, colorless, flaky growth on bottom of flask or tube.

Aerial mycelium: None.

Soluble pigment: None.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with starch as a source of carbon; none, with saccharose and glycerin.

2. *Proteolytic action:* Fair in milk and gelatin.

3. *Change of reaction:* With NaNO$_3$ as a source of nitrogen, the medium turns alkaline for all sources of carbon used; faintly alkaline on milk.

4. *Inversion of sugar:* None.

5. *Diastatic action:* Very good amylolytic power, but weak saccharogenic power; 1 per cent starch all reduced to the erythro-reaction in 14 days; very good by tube and plate methods, but hydrolysis of the starch is in all cases incomplete, reduced chiefly to the erythro-reaction.

6. *Growth on cellulose:* Faint to fair, particularly on paper in synthetic solution; no ring formation on plate.

Hab. Isolated from California upland soil.

A. violaceus-niger described originally (45) and several strains of the chromogenus group were found on further cultivation to be closely related to this species and are therefore omitted here. The *A. violaceus-caesari*, although a distinct species resembles in certain respects the chromogenus forms.

Actinomyces violaceus-ruber Waksman and Curtis

I. MORPHOLOGY.

1. Spirals.

Synthetic agar: Numerous, of the open type.

Dextrose agar: Short, imperfect spirals; both spirals and straight mycelium breaking up into spores; spirals dextrorose.

2. Conidia.

Synthetic agar: Oval and rod-shaped, 0.8 to 1.5 x 0.7 to 1.0 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar, 15 days.

Growth: Thin, spreading, growing deep into medium, uncolored at first, soon changing to red and then blue.

Aerial mycelium: Thin, powdery, covering all colony, at first white, later turning mouse-gray (Rdg. LI, 15′′′′′).

Soluble pigment: A red soluble pigment is first produced which changes to cyanine blue (Rdg. IX, 51-m). The production of the pigment and successive changes can be readily explained by the change in reaction of the medium. The organism produces a soluble pigment, which acts as an indicator; it changes from red to blue at P$_H$ 7.4–7.6. The synthetic agar has a reaction of P$_H$ 7.0, therefore the pigment when produced is red. The medium becomes more alkaline as a result of the growth of the organism; when the reaction changes

to P_H 7.4–7.6, the red pigment changes to blue. The rapidity of the change of pigment differs with the strains of the organism, depending probably on the rapidity of alkali production. When studied in solution, the change of color is not very sharp, but somewhat gradual, varying from red to red brown and blue. This pigment is only one of two or even three pigments present in the mixture; it may therefore often be obscured.

2. Calcium malate-glycerin agar, 15 days.

Growth: Thin, spreading, penetrating to some extent into the medium; rose red in color.

Aerial mycelium: Thin, powdery, ash-gray, with white edge; abundant, all over growth.

Soluble pigment: None in 15 days; red pigment slowly spreading appears in 35 days, the lack of blue pigment is no doubt due to the fact that this medium does not turn alkaline to the indicator produced by the organism.

3. Glucose agar, 15 days.

Growth: Spreading, more on surface than into medium, edge regular; of a brick-red color, later turning almost black.

Aerial mycelium: Thin, cottony, ash-gray, with white edge; abundant all over growth.

Soluble pigment: None at first: faint red to reddish brown pigment appears in 35 days.

4. Nutrient agar.

Growth: At first (4 days) white, later becoming red, with white margin.

Aerial mycelium: White, appearing only in 10–12 days.

Soluble pigment: Blue, spreading very slow.

5. Blood agar, 37°.

Growth: A slightly elevated, crumpled, spreading, red growth appears early (2–3 days), surface glistening

Aerial mycelium: None.

Soluble pigment: Faint brown, limited to reverse of growth only.

Hemolysis: Strong.

6. Blood serum, 37°.

Growth: Thin, brownish spreading smear in 3–4 days, later turning red; surface glistening.

Aerial mycelium: None.

Soluble pigment: Soluble red pigment (7 days).

Liquefaction: None.

7. Egg-media, 37° (also at 25°, although growth is somewhat slower).

Growth: Thin, spreading brown smear in 5–6 days.

Aerial mycelium: White, all over growth, with a bluish tinge, developing in 2 weeks.

Soluble pigment: Blue, often none.

8. Starch plate, 12 days.

Growth: Spreading, pink-colored.

Aerial mycelium: Powdery, ash-gray color, all over surface of growth; the marginal zone is often white.

Soluble pigment: None.

Enzymatic zone: 6–8 mm. wide.

9. Potato plug.

Growth: Small brownish colonies appearing in 3–4 days; in about 2 weeks growth is found to be abundant, folded, of a grayish color.

Aerial mycelium: Abundant, white, with ash-gray patches in 7–8 days; bluish tinge in 2 weeks.

Color of plug: Blue in 4–5 days.

10. Carrot.

Growth: Abundant, spreading, much folded, lichnoid, cream-colored at first, later turning brownish.

Aerial mycelium: Gray, powdery all over surface, with shade of pink developing in 15 days.

Color of plug: Bright pink around growth, with dark brown pigment spreading over the plug.

11. Gelatin, 18°, 18 days.

Growth: Spreading, dense, cream-colored at first, the underlying part changing to pink or blue depending on reaction of gelatin to start with; quite often the color remains grayish.

Aerial mycelium: White or none at all, when the flaky growth drops to the bottom of the liquefied portion.

Soluble pigment: None, sometimes blue.

Liquefaction: Slow to medium; in presence of starch it is more rapid; 1½ cm. of depth of tube liquefied in 30 days.

12. Synthetic solution, 15 days.

Growth: Numerous, small, round colonies throughout the medium.

Aerial mycelium: Scant, gray.

Soluble pigment: Red changing to blue.

13. Milk, 37°, 15 days.

Growth: Gray with shade of red or blue pigmented ring on surface of milk in contact with glass.

Coagulation: Usually none; often it takes place in 9-10 days, followed by a very slow peptonization; the clot when produced is soft.

Hydrolysis: 12-15 days, complete; hydrolyzed portion has a pinkish tinge.

Change of reaction: Distinctly alkaline (5).

14. Glucose broth, 15 days.

Growth: Solid grayish ring on surface, close to tube, none throughout medium.

Aerial mycelium: White.

Soluble pigment: Blue.

15. Utilization of different carbon compounds.

Arabinose	1	*Dextrose*	5	*Lactose*	5
Glycerin	3	*Saccharose*	3	*Starch*	5
Cellulose	2-3	*Organic acids*	0-1	*Maltose*	3-4
Mannite	4				

16. Utilization of different nitrogen compounds.

Ammonium sulfate	0	*Ammonium carbonate*	0
Sodium nitrite	3	*Acetamide*	2
Sodium nitrate	1	*Leucin*	3
Glycocoll	3-4	*Casein*	3-4
Asparagin	3-4	*Fibrin*	3
Egg-albumin	2	*Urea*	1-2
Peptone	3-4		

III. Biochemical Features

1. *Nitrite formation:* Excellent with nearly all sources of carbon.

2. *Proteolytic action:* Good on milk and on gelatin.

3. *Change of reaction:* With NaNO₃ as source of nitrogen, the medium is made alkaline with the following sources of carbon: salts of organic acids, maltose, dextrose, lactose, etc. When grown on acid gelatin, the reaction changes to

alkaline (from P_H 6.2 to P_H 8.0) both in presence and absence of starch; distinctly alkaline in milk; alkaline glucose broth made acid (P_H changed from 7.9 to 6.6).

6. *Inversion of sugar:* Usually positive.

5. *Diastatic action:* Good; 1 per cent starch not used up entirely in 14 days; good by tube and plate methods.

6. *Growth on cellulose:* Fair to good, depending on method used; cellulose used fairly well; clear zone formed on cellulose plate.

Hab. Isolated from Iowa and California adobe soils.

Actinomyces viridochromogenus Krainsky 1914, p. 684, emend.
Waksman and Curtis

Isolated numerous times from different soils; strains differ chiefly in vigor of growth on artificial media; the following description is based chiefly on one strain (101).

I. MORPHOLOGY.
    1. Spirals.
        Synthetic agar: Abundant, regular, 3–5 μ, in diameter.
        Starch: Numerous, perfect, of an open type, 3–6 μ in diameter, occurring as side branches on long mycelia and terminal.
    2. Conidia.
        Synthetic agar: Short ovals to spheres, 1.25 x 1.25–1.5 μ.
        Starch: Spherical to oval 1–1.25 x 1.0–1.5 μ.

II. CULTURAL CHARACTERISTICS..
    1. Synthetic agar.
        Growth: Spreading, cream-colored with dark center at first, later all surface growth becomes dark green; reverse yellowish to light cadmium (Rdg. IV, 19)..
        Aerial mycelium: Abundant, covering center first, then spreading all over surface; white, changing to light geladine green (Rdg. XLVII, 35''''–d).
        Soluble pigment: None.
    2. Calcium malate-glycerin agar.
        Growth: Thin, spreading, developing deep into medium; light gray at first, later changing to dark-green and almost black.
        Aerial mycelium: Thin, powdery, all over surface, white at first, later becoming pale dull glaucous-blue (Rdg. XLII, 41'''–f).
        Soluble pigment: None.
    3. Glucose agar.
        Growth: Spreading, abundant, developing chiefly on surface, partly into the medium, wrinkled, gray at first, later becoming black.
        Aerial mycelium: White at first, later becoming greenish-blue.
        Soluble pigment: Faint brown, with greenish tinge.
    4. Nutrient agar, 15 days.
        Growth: Abundant, restricted, gray growth, with greenish tinge.
        Aerial mycelium: Powdery, white, with shade of green all over surface of growth.
        Soluble pigment: Dark brown, spreading.
    5. Blood agar, 37°, 15 days.
        Growth: Dark gray, crumpled colonies, later turning brown.
        Aerial mycelium: Scant white.
        Soluble pigment: Dark, spreading.
        Hemolysis: None.

6. Blood serum, 37°.

  Growth: Small, glossy, brownish colonies in 4–5 days.

  Aerial mycelium: White, with dark center.

  Soluble pigment: Spreading, dark zone around growth.

  Liqueaction: None.

7. Egg-media, 37°.

  Growth: Extensive, spreading, brown growth in 5–6 days.

  Aerial mycelium: White, with greenish patches in 5–6 days; pinkish tinge, when culture becomes older (15 days).

  Soluble pigment: Dark all through medium.

8. Starch plate, 12 days.

  Growth: Round, spreading, yellowish.

  · *Aerial mycelium:* Thin, greenish to gray, with zone formation.

  Soluble pigment: None.

  Ensymatic zone: Scant (2 mm. in 11 days).

9. Potato plug.

  Growth: Abundant, gray-brown growth appears early (24 hours).

  Aerial mycelium: Abundant, white, all over growth (3–4 days), developing a greenish tinge (15 days), which changes to pinkish with age of culture (30 days.)

  Color of plug: Black, spreading.

10. Carrot.

  Growth: Restricted at first, later spreading, folded, cream-colored with dark shade developing from center of growth.

  Aerial mycelium: Cottony, abundant, white, with shade of green.

  Color of plug: Dark brown.

11. Gelatin, 18°, 30 days.

  Growth: Colonies cream-colored, becoming greenish.

  Aerial mycelium: Green, with yellow tinge.

  Soluble pigment: Brown.

  Liquefaction: Slow.

12. Synthetic solution.

  Growth: Small flakes on side of tube; floating colonies; surface of a characteristic green color.

  Aerial mycelium: Green.

  Soluble pigment: None to shade of brown.

13. Milk, 37°. Soluble brown pigment.

  Growth: Dark brown surface growth with shade of green in the form of a ring at 25°; none at 37°.

  Coagulation: 5–6 days.

  Peptonization: Begins in 6 days, proceeds rapidly and in 12 days coagulum is all (imperfectly) digested; digestion much slower at 25°.

  Hydrolysis: May take place instead of coagulation, particularly at 25°.

  Change of reaction: Faintly alkaline (1).

14. Glucose broth, 12 days.

  Growth: Dense solid ring on surface, in contact with glass, also small masses on surface of liquid; brownish, turning dark green in color.

  Aerial mycelium: Thin, powdery, white to faint greenish.

  Soluble pigment: Faint greenish to deep brown spreading downward.

15. Utilization of different carbon compounds.

Arabinose 3	*Dextrose* 4	*Lactose* 3
Glycerin 2	*Saccharose* 2	*Maltose* 5
Cellulose 1–3	*Mannite* 3	*Starch* 3
Organic acids ...... 1–2 (lactate, malate, and tartrate)		

16. Utilization of different nitrogen compounds.

Ammonium sulfate	0	*Ammonium carbonate*	0
Sodium nitrite	1–3	*Acetamide*	1
Sodium nitrate	2	*Leucin*	3
Glycocoll	3–5	*Casein*	4–5
Asparagin	3	*Fibrin*	3
Egg-albumin	3	*Urea*	3
Peptone	4		

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Scant, with sucrose and glycerin as sources of energy, much better with starch.
2. *Proteolytic action:* This organism has a rather weak proteolytic action; fair on milk and gelatin; faint to fair on the proteins (fibrin, egg-albumin, casein).
3. *Change of reaction:* Alkaline with NaNO$_3$ as a source of nitrogen and with different carbon compounds as sources of carbon; faintly alkaline in milk; faint acidity with different proteins and amino acids as sources of nitrogen and glycerin as a source of carbon; faint acidity in glucose broth.
4. *Inversion of sugar:* None.
5. *Diastatic action:* Fair action in a 1 per cent starch solution; scant action on plate, zone 2 mm. in 11 days.
6. *Action on cellulose:* Usually good, but there was no zone formation on plate.

Hab. Isolated from New Jersey garden, California, Oregon adobe, Porto Rico and Texas soils.

Actinomyces 104

I. MORPHOLOGY.
1. Spirals.
Numerous, of the open type, on synthetic agar; few spirals observed on dextrose agar and glycerin.

II. CULTURAL CHARACTERISTICS. This organism is characterized by a very scant growth on the synthetic agar.
1. Synthetic agar.
Growth: Colorless, spreading, chiefly deep into the medium.
Aerial mycelium: Very thin, white; turning later grayish.
Soluble pigment: None.
2. Calcium malate-glycerin agar.
Growth: Medium, spreading chiefly on surface; edge myceloid; cream-colored with brownish shade.
Aerial mycelium: None.
Soluble pigment: None.
3. Glucose agar.
Growth: Abundant, spreading growth, chiefly on surface, also penetrating below surface; edge myceloid, color of surface growth brown with wide colorless margin.
Aerial mycelium: Thin, powdery, all over surface, with narrow margin, white-colored.
Soluble pigment: Brown.
4. Nutrient agar.
Growth: Cream-colored, wrinkled, only on surface.
Aerial mycelium: None.
Soluble pigment: None.

5. Blood serum, 37°.
    Growth: Glossy, wrinkled, gray growth.
    Aerial mycelium: None.
    Soluble pigment: None.
    Liquefaction: Starts in 15 days.
6. Egg-media.
    Growth: Thin, spreading, fine net-work, cream-colored with green tinge.
    Aerial mycelium: None.
    Soluble pigment: None.
7. Starch plate, 25°, 16 days.
    Growth: Thin, spreading, cream-colored.
    Aerial mycelium: None or a few grayish thin patches.
    Enzymatic zone: 5–6 mm. wide; hydrolysis of the starch is incomplete.
8. Potato plug.
    Growth: Abundant, much wrinkled, greenish at first (4 days), later becoming
        black with yellowish margin.
    Aerial mycelium: None.
    Color of plug: Faint black zone around growth appears late (15 days).
9. Carrot, 25°, 22 days.
    Growth: Abundant, spreading, much folded, brownish.
    Aerial mycelium: Scant, white patches.
    Color of plug: Brown shade appears late (22 days).
10. Gelatin, 18°, 35 days.
    Growth: Cream-colored flakes on bottom of liquefied portion.
    Aerial mycelium: None.
    Soluble pigment: None.
    Liquefaction: Rapid (3 cm. of depth of gelatin liquefied in tube in 35 days).
11. Synthetic solution.
    Colonies: Scant flaky growth on bottom of tube.
    Aerial mycelium: None.
    Chromogenesis: None.
12. Milk, 37°.
    Growth (25°): Pinkish surface ring in contact with glass.
    Coagulation: 8–12 days.
    Peptonization: Begins in 10–12 days and is nearly all completed 20–25 days.
    Change of reaction: Fairly (2) to distinctly alkaline (3).
13. Glucose broth 25°, 12 days.
    Growth: Thin, cream-colored pellicle on surface, some flakes on bottom.
    Aerial mycelium: None.
    Soluble pigment: None.
14. Utilization of different carbon compounds (NaNO₃ as source of nitrogen).

Arabinose	0	*Dextrose*	1	*Lactose*	1
Glycerin	1	*Saccharose*	1	*Maltose*	2
Cellulose	1	*Mannite*	1	*Starch*	1
Organic acids	0–1				

III. BIOCHEMICAL FEATURES.
1. *Nitrite formation:* Scant, though positive, with most sources of carbon.
2. *Proteolytic action:* Good on gelatin, both in presence and absence of starch; fair on
    milk and glucose broth.
3. *Change of reaction:* No change, slight acidity on alkalinity with NaNO₃ as source
    of nitrogen and different sources of carbon; faint alkalinity in gelatin and glu-
    cose broth and in milk.

4. *Inversion of sugar:* None.
5. *Diastatic action:* Fair in plate; good by tube method.
6. *Growth on cellulose:* None or very scant.
Hab. New Jersey orchard soil, 8 inches deep.

Actinomyces 145

I. MORPHOLOGY.
    1. Spirals.
        None on the media studied; hyphae usually coarse, branching, with a tendency
            to curl.
    2. Conidia.
        Oval-shaped to elliptical spores.

II. CULTURAL CHARACTERISTICS.
    1. Synthetic agar.
        Growth: Thin, colorless, spreading, developing deep into the medium.
        Aerial mycelium: Thin, gray.
        Soluble pigment: None.
    2. Calcium malate-glycerin agar.
        Growth: Scanty, colorless, raised in center, uneven edge.
        Aerial mycelium: None.
        Soluble pigment: None.
    3. Glucose agar.
        Growth: Good, spreading growth, cream-colored chiefly on surface, penetrating
            to a slight extent into the medium, center raised, edge myceloid.
        Aerial mycelium: Abundant all over surface, pale mouse-gray (Rdg. LI, 15′′′′′–d)
            with white patches.
        Soluble pigment: None.
    4. Nutrient agar.
        Growth: Thin, cream-colored.
        Aerial mycelium: Gray.
        Soluble pigment: None.
    5. Blood serum, 25°, 20 days.
        Growth: Faint cream-colored spots.
        Aerial mycelium: None.
        Soluble pigment: None.
        Liquefaction: None.
    6. Egg-media, 25°, 20 days.
        Growth: Abundant, spreading, dark brown, appearing late.
        Aerial mycelium: None.
        Soluble pigment: Narrow purplish zone around growth.
    7. Potato plug, 25°, 15 days.
        Growth: Small restricted colonies, gray at first (4 days), later (15 days) becom-
            ing dark.
        Aerial mycelium: None at first, traces of white appearing in 15 days.
        Color of plug: Black.
    8. Carrot, 25°, 22 days.
        Growth: Scant, spreading, raised in center, cream-colored.
        Aerial mycelium: None.
        Color of plug: Brown zone only in 22 days.

9. Starch plate, 25°, 15 days.

> *Growth:* Rapidly spreading, cream-colored, with yellow reverse.
>
> *Aerial mycelium:* Light buff-gray.
>
> *Enzymatic zone:* Wide (12–15 mm.), hydrolysis of starch imperfect.

10. Gelatin, 18°, 35 days.

> *Growth:* Cream-colored to brownish, spreading chiefly on side of tube.
>
> *Aerial mycelium:* Abundant, white, covering all growth.
>
> *Soluble pigment:* Brown in liquefied portion.
>
> *Liquefaction:* Slow, presence of starch seems to further it.

11. Synthetic solution.

> *Growth:* None.

12. Milk, 37°.

> *Growth* (25°): Dark brown surface zone, abundant white aerial mycelium.
>
> *Coagulation:* 6–7 days.
>
> *Peptonization:* Begins soon after coagulation, advances very slowly, and is not completed in 50 days.
>
> *Change of reaction:* Unchanged.

13. Glucose broth, 25°, 12 days.

> *Growth:* Thin, dark gray pellicle on surface consisting of round colonies.
>
> *Aerial mycelium:* None to a thin ash-gray layer.
>
> *Soluble pigment:* Deep brown, spreading downward.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with glycerin as a source of carbon.
2. *Proteolytic action:* Scant on milk, fair to good (in presence of 1 per cent starch) on gelatin.
3. *Change of reaction:* Unchanged or slightly acid in gelatin, unchanged in milk, distinctly acid in glucose broth.
4. *Inversion of sugar:* None.
5. *Diastatic action:* Good on plate, chiefly to the erythro reaction.
6. *Growth on cellulose:* None.

Hab. Hawaiian pineapple soil.

Actinomyces 206

I. MORPHOLOGY.

1. Spirals.

> None on most media; the growth takes place in small individual clumps with straight, branching hyphae; a few long, open spirals present on the calcium malate agar.

2. Conidia.

> *Synthetic agar:* Spherical and oval, 0.9 to 1.1 x 0.9 to 2.0 μ.

II. CULTURAL CHARACTERISTICS.

1. Synthetic agar.

> *Growth:* Abundant, spreading, developing deep into the medium; color of growth yellow to olive-ocher (Rdg. XXX, 21″); reverse yellow to almost black.
>
> *Aerial mycelium:* Mouse-gray to light drab (Rdg. XLVI, 17″″–C).
>
> *Soluble pigment:* None.

2. Calcium malate-glycerin agar.

Growth: Thin growth penetrating to some extent into the medium, edge myceloid, color deep colonial buff (Rdg. XXX, 21"b).

Aerial mycelium: Thin, over large part of surface; minute droplets of water forming a silvery zone on surface of it; color violet-gray (Rdg. LII, 59''''') with yellowish margin.

Soluble pigment: None.

3. Glucose agar.

Growth: Abundant, restricted on surface and penetrating to some extent into the medium; center raised; edge entire; color as before.

Aerial mycelium: Covering only portions of surface, color as on calcium malate.

Soluble pigment: None in 15 days, in 35 days faint yellow.

4. Nutrient agar.

Growth: White smear, surface glistening.

Aerial mycelium: None.

Soluble pigment: None.

5. Blood serum, 37°.

Growth: Spreading, glistening, cream-colored smear.

Aerial mycelium: None.

Soluble pigment: None.

Liquefaction: Medium, begins in 6–7 days.

6. Egg-media, 37°.

Growth: Thin, spreading, gray growth.

Aerial mycelium: Thin white patches.

Soluble pigment: None.

7. Starch plate.

Growth: Thin, spreading, yellowish green in color.

Aerial mcyelium: Dark gray.

Enzymatic zone: Good (3).

8. Potato plug.

Growth: Abundant, much wrinkled, elevated, gray at first (4 days), later (8 days) turning sulfur-yellow on edge; in 30 days, the plug is nearly all destroyed (shrivelled up) by the growth of the organism.

Aerial mycelium: Abundant all over growth, light yellow with mouse gray patches.

Color of plug: Unchanged.

9. Carrot, 25°, 22 days.

Growth: Abundant, spreading, cream-colored.

Aerial mycelium: Abundant, powdery, gray colored, all over growth.

Color of plug: Unchanged.

10. Gelatin, 18°, 35 days.

Growth: Cream-colored flaky mass on bottom of liquefied portion; in exposed part, close to glass, yellow.

Aerial mycelium: Thin white on yellow exposed portion of growth.

Soluble pigment: None.

Liquefaction: Rapid (4 cm. in 35 days).

11. Synthetic solution.

Growth: Scant growth to almost none. When glycerin is substituted for saccharose there is formed an abundant yellowish brown pellicle on surface of liquid, with a yellow aerial mycelium and a yellow soluble pigment.

12. Milk, 37°.

Growth (25°): Faint pinkish growth, accompanied by hydrolysis of milk.

Coagulation: 4–5 days.

Peptonization: Begins in 4–5 days and proceeds very rapidly so that it is all completed in 10–12 days.

Change of reaction: Faintly (1) to fairly alkaline (2).

13. Glucose broth, 25°, 12 days.

Growth: Wide, thick ring on surface in contact with glass; color sulfur-yellow, often shade of orange is produced.

Aerial mycelium: Thin, ash-gray, all over growth.

Soluble pigment: None or faint sulfur-yellow pigment.

III. BIOCHEMICAL FEATURES.

1. *Nitrite formation:* Fair, with starch and glycerin as sources of carbon.

2. *Proteolytic action:* Very good in milk and glucose broth; excellent on gelatin, but only good in the presence of 1 per cent starch.

3. *Change of reaction:* Fairly alkaline in milk and glucose broth; distinctly alkaline in acid gelatin, in absence and presence of starch.

4. *Inversion of sugar:* Positive, often negative.

5. *Diastatic action:* Good, no starch left in a 1 per cent solution at the end of 14 days; good on plate, enzymatic zone 12–15 mm. wide.

6. *Growth on cellulose:* Good, with paper as the only source of carbon.

Hab. Isolated from Oregon, California adobe, and Maine Aroostook soils.

COMPARATIVE CULTURAL AND BIOCHEMICAL STUDIES

Many species have shown, in the cultural and biochemical studies, unique differences which assist in their separation, but the most important results are obtained when these organisms are separated into groups, which resemble closely one another in their metabolism. The actinomycetes vary in their metabolism as much as any other large complex group of forms of life, and a distinct separation into species is almost next to impossible, because new forms will be found which will tend to bring two distinct species together; it is therefore much more advisable, in the study of these organisms, to separate them into species-groups where the species resembling one another in their metabolism would fall. A number of the descriptions of the species given above are based not upon a single organism but upon a group of closely related forms.

Several simple synthetic media were used so that the information could be readily duplicated and a better insight into the metabolism of the organisms could be obtained. Many of the results reported were repeated two and three times, and any differences that occurred on the following observations were carefully noted. As stated above, most of these organisms were described 4 years ago, soon after their isolation from natural substrata; after they were grown for this period of time on artificial, usually synthetic, media, some organisms have shown cultural differences distinctive from those obtained 4 years previously, and these differences also were carefully noted. In inoculating cultures for the work, aerial mycelium (including conidial) was used where possible, otherwise a piece of the growth was transferred by means of a

sterile platinum loop or heavy needle, the point of which was flattened. Where an organism failed to grow on inoculation upon a certain medium, the inoculation was repeated on another tube or often on a fresh lot of medium. It is quite possible that even with these precautions certain differences escaped attention, but these would be usually of limited value in establishing the cultural identity of an organism, and it is believed that the cultural and biochemical studies reported represent fairly accurately the comparative reactions of the organisms used.

For convenient comparison of the species, the data are tabulated below. A complete discussion of the metabolism of actinomycetes, including complete tables for this group of organisms, has recently been published elsewhere (43). Only several of the tables are repeated here for purposes of comparison, but, for a complete historical review on the metabolism of these organisms and a discussion of the data, the reader is referred to the other papers.

UTILIZATION OF CARBON COMPOUNDS AS SOURCES OF ENERGY

In all the studies of utilization of carbon compounds, 3 per cent of the sources of carbon were added to the synthetic solution with 0.2 per cent of NaNO₃ as the only source of nitrogen.

Arabinose. Out of 23 organisms tested for the utilization of arabinose as the only source of carbon, *A. aureus, A. poolensis, A. albosporeus, A. lipmanii, A. verne, A. bobili, A. reticulus-ruber, A.* 128, *A.* 205, *A.* 168, *A. asteroides* and *A.* 104 refused to grow at all; *A. roseus* and *A. ruber* produced only a few flakes at the bottom of the tube; *A. violaceus-ruber* and *A. violaceus-caesari* a few minute colonies through the medium or on the surface; *A. albus* made a fair surface growth; *A. viridochromogenus, A. griseus, A. exfoliatus, A. fradii, A. scabies* and *A. diastaticus* made a good to very good surface growth with a fair to abundant aerial mycelium. The organisms that made a good growth affected a change in the hydrogen-ion concentration of the medium towards alkalinity. *A. violaceus-caesari* produced a blue soluble pigment; *A. griseus, A. scabies* and *A. exfoliatus* a yellow to brown soluble pigment. *A. violaceus-ruber, A. ruber, A. roseus* and *A. viridochromogenus* reduced the nitrate to nitrite in appreciable amounts, while some cultures (*A. fradii, A. lipmanii*) contained mere traces of nitrites (*A. madurae, A. hominis* and *A. bovis* were used only in a few instances in the carbon studies).

Dextrose. Dextrose proved to be one of the best sources of carbon for the actinomycetes. Out of the 26 organisms inoculated upon the solution, only *A. verne, A. poolensis, A.* 205 and *A.* 104 made a scant flaky growth through the medium or on the bottom, with no change of reaction or nitrite production (traces with *A. verne*). Most of the other organisms (*A. violaceus-ruber, A. exfoliatus, A.* 168, etc.) made a very good to excellent growth, usually in the form of surface colonies or in the form of a heavy pellicle, often developing out of the colonies growing together, and a good characteristic, aerial mycelium.

A. griseus and *A.* 168 produced a golden pigment, while *A. viridochromogenus*, *A. scabies* and *A. exfoliatus* produced a brown soluble pigment. The change of reaction was distinctly towards the alkaline for the organisms that produced a good growth. *A. violaceus-ruber*, *A. roseus*, *A. reticulus-ruber* and *A. asteroides* produced maximum quantities of nitrites, a few other species only traces or fair quantities, while most organisms did not produce any nitrite in the presence of dextrose.

Maltose. Maltose was found to be also a very good source of carbon. Out of the 26 organisms studied, only *A. poolensis*, *A. albosporeus*, *A. madurae* and *A. bovis* made a scant flaky growth on the bottom of the tube. Most of the organisms made a good to very good growth with this source of carbon, but only *A. viridochromogenus* made an excellent growth, accompanied by a distinct change in reaction towards acidity. The growth is usually on the bottom of the tube, but quite often (*A. griseus*, *A. diastaticus*, *A.* 168) on the surface of the liquid with an abundant aerial mycelium. The change in reaction was in a few cases toward acidity (*A. viridochromogenus*, *A. violaceus-caesari*, *A. albus*, and *A.* 104), in most cases unchanged or changed to distinctly alkaline (P_H changed for *A. violaceus-ruber* from 6.2 to 7.2, *A. griseus* to 7.6, *A. diastaticus* to 8.0, *A. hominis* to 7.4, *A. reticulus-ruber* to 7.1, *A.* 205 to 7.2, *A. ruber* to 7.0.) The nitrite production was good for a number of organisms, *A. violaceus-ruber* producing a maximum quantity.

Saccharose. Saccharose is a rather poor source of carbon for many actinomycetes, because not all the species are able to invert this sugar. It is quite possible that some organisms, on continued cultivation on artificial media, begin to produce some inverting enzyme, as was observed in a few cases. The growth in solution was usually scant to fair, in the form of flakes on the bottom of the tube or as colonies throughout the medium; only *A. ruber* and *A. exfoliatus* produced an excellent growth on saccharose in solution. The change of reaction was either faintly acid (*A. asteroides*, *A. scabies*, and *A. exfoliatus*), faintly alkaline or distinctly alkaline (*A. griseus*, *A. diastaticus*, *A. aureus*, *A. reticulus-ruber*, *A. madurae*, *A. bobili*, *A. viridochromogenus* and *A.* 104). The reduction of nitrate to nitrite is usually none or scant, excellent only for *A. violaceus-ruber* and *A. bovis*, and good for a few others (*A. asteroides*, *A. verne*, *A. albus*, and *A. reticulus-ruber*). Of course these comparisons hold true, in the case of this source of carbon as well as the others, only in liquid media; on agar media much better growth of most species is obtained.

Lactose. Lactose is a good source of carbon for most species. Out of nearly 30 species tested, only *A. violaceus-caesari* and *A.* 104 produced a scant flaky growth on the bottom of the tube and only *A. violaceus-ruber* an excellent growth. Most organisms produced a good to very good growth, usually in the form of colonies on the surface of the liquid or in the form of a heavy pellicle accompanied by fair to abundant aerial mycelium. *A. scabies*, *A. exfoliatus* and *A. viridochromogenus* produced a brown soluble pigment and *A. violaceus-ruber* a blue pigment. The change of reaction was usually toward

alkaline. Most organisms produced no nitrite or mere traces; only *A. bovis* and *A. violaceus-ruber* produced a maximum quantity, *A. verne* and *A. viridochromogenus* "very good" amounts of nitrites.

Glycerin. Glycerin favored a good development of most species, although fewer organisms made as abundant a growth as on dextrose and starch in solution. It is interesting to note that *A. poolensis* that made a scant growth on most other carbon compounds made a very good growth on glycerin. *A. verne*, *A.* 128, *A. albosporeus*, *A.* 104 and *A.* 205 produced only a scant growth. *A. griseus*, which made a heavy growth on most carbon compounds, except saccharose and the salts of organic acids, made a rather limited growth on glycerin. A soluble pigment was formed only in the culture of *A. exfoliatus*. The reaction of the solution was either left unchanged or changed to faintly acid or alkaline; *A. asteroides* and *A. violaceus-ruber* caused a distinct change in the glycerin medium toward acidity. Nitrites were produced by most organisms, but in quantities not larger than mere traces; only *A. violaceus-ruber*, *A. viridochromogenus*, *A. lipmanii*, *A. bovis*, *A. hominis* and *A. asteroides* produced appreciable quantities of nitrites.

Mannite. Mannite was found to be a good source of carbon only for about half of the actinomycetes tested. *A. aureus* was the only species that made an excellent growth, while *A. poolensis*, *A. violaceus-caesari*, *A.* 128, *A. roseus*, *A. reticulus-ruber*, *A. bobili*, *A. asteroides*, *A.* 104, and *A.* 205 made a scant growth. The hydrogen-ion concentration was left unchanged or usually changed to alkaline, notably *A. griseus* changing the reaction from P_H 7.2 to 7.7, *A. scabies* to 7.6 and *A. luteus* to 7.5. Very few cultures changed the medium to slightly acid; among these *A. asteroides* occupies the most prominent place, changing the P_H values from 7.2 to 6.5. A soluble brownish pigment was produced only by *A. exfoliatus* and *A. viridochromogenus*. The reduction of nitrates to nitrites was scant or entirely absent for most organisms; *A. violaceus-ruber*, *A. reticulus-ruber*, *A. verne* giving the maximum quantities, while *A. albosporeus* and *A. griseus* gave only good reduction.

Starch. Starch was found to be the best source of carbon of all the compounds tested for most of the 26 species used. Only *A. verne* and *A.* 104 that made a rather scant growth on all other carbon sources gave a scant flaky growth on starch as the only source of carbon and *A. asteroides* made hardly any growth at all; most other organisms made a good to very good· growth usually in the form of colonies or a heavy pellicle on the surface of the liquid and accompanied by a good aerial mycelium. The change in reaction of the medium was usually none or (more often) toward distinct alkalinity. So, for example, *A. ruber* changed the hydrogen-ion concentration of the medium (P_H values) from 7.0 to 7.6, *A. diastaticus*, *A. albosporeus* and *A. viridochromogenus* to 7.5, etc. Some few organisms produced a faint acidity in the medium, *A.* 128 and *A.* 104 changing the P_H values from 7.0 to 6.8. A brown soluble pigment was produced by *A. lipmanii* and *A. exfoliatus*, while a fair brown (golden) pigment by *A.* 128 *A. griseus*, *A. fradii*, *A.* 168 and

A. 205. The reduction of nitrates to nitrites was scant or none for most of the species that gave weak nitrite accumulation with other carbon compounds, but the active nitrate-reducing organisms usually allowed large quantities of nitrites to accumulate in the medium. The high average for nitrate reduction with starch as a source of carbon should not therefore be looked upon as an indication that more organisms reduce nitrates with this source of carbon than with any other, but that those that reduce nitrates readily do it even more actively with starch as a source of carbon, which as was shown elsewhere (43) is explained by the metabolism of the organisms. Starch as a good source of energy allows a better development of the organisms and this is accompanied by a greater reduction of nitrates in the medium. *A. violaceus-ruber, A. roseus, A. lipmanii* and *A. reticulus-ruber* gave the "excellent" reduction of nitrates.

Starch is readily utilized by most actinomycetes because most of them produce an active diastatic ferment which hydrolyzes the starch actively, thus making it readily available as a source of carbon. This was shown to hold true both by the plate and the tube methods, as seen in table 7. A complete discussion on the hydrolysis of starch by actinomycetes will be found in the paper on the "Carbon Metabolism of Actinomycetes" (43).

Inulin. Out of 12 species tested with this source of carbon, only *A. reticuli* made a scant growth, while *A. ruber* and *A. bovis* made a good to very good growth. The other 9 species made only a fair growth; this was usually in the form of colonies on the bottom of the tube or a thin pellicle on the surface, covered with a thin aerial mycelium. With $NaNO_3$ as a source of nitrogen, 9 out of the 12 cultures changed the medium from nearly neutral (P_H 6.8) to distinctly alkaline (P_H 7.6–8.1), only *A. exfoliatus, A. ruber,* and *A. diastaticus* produced no change in reaction of faint acidity. Only *A. exfoliatus* produced a yellowish soluble pigment.   ·

Cellulose. The utilization of cellulose by actinomycetes was tested by several different methods, but none of them proved very satisfactory, as was shown above. This can be further emphasized by the fact that the different methods did not check up with one another; some organisms that were found to use cellulose readily by one method were not found to do so by another; this is no doubt due to the difference in the form of cellulose used in the different methods. The simplest and easiest way of testing the cellulose utilization by actinomycetes is the introduction of a piece of filter paper into the synthetic solution, without any other source of carbon. Those organisms that can use cellulose as the only source of carbon will be found to form colonies or flaky growth on the paper and then throughout the medium; this is also accompanied by a change in the reaction of the medium and the reduction of nitrates to nitrites. As was shown elsewhere (43), the reduction of nitrates and the changes in the hydrogen-ion concentration of the medium can be taken, for many organisms, as an index of their utilization of the proper carbon compound; this is particularly important in the study of the cellulose utilization by

the actinomycetes as the only source of carbon, since not only the actual growth obtained, but also the change in the hydrogen-ion concentration and the reduction of nitrates can be recorded. Out of the 30 or so species studied on cellulose, many produced a scant flocculent growth on the bottom of the tube or none at all. *A. violaceus-ruber*, *A. violaceus-caesari*, *A. scabies*, *A. ruber*, and *A.* 205 made a fair growth accompanied by a faint change in reaction toward alkalinity and traces of nitrites in some instances (fair with *A. violaceus-ruber*); *A. verne*, *A.* 168 made a good growth, *A. bobili* and *A. reticulus-ruber* made a very good growth. *A. asteroides*, with a scant growth, was the only organism that changed the reaction of the medium to distinctly acid (from P_x 7.0 to 6.5). *A. ruber*, *A. albus*, *A. exfoliatus*, *A. aureus*, *A. violaceus-ruber*, *A. scabies* and *A. bobili* made a very good growth on the cellulose plate, most of them producing a clear ring around the colony; *A. albus*, *A. ruber*, *A. viridochromogenus* and *A. lipmanii* made the best growth upon filter paper by using the method of Krainsky (22).

Organic acids. Several sodium salts of organic acids were studied as sources of carbon for actinomycetes (30 gm. per liter), namely, the salts of acetic, lactic, malic, tartaric and oxalic acids. The growth of most of the organisms on these acids was limited to a few flakes on the bottom of the tube, accompanied in most instances by a distinct alkalinity and none or only scant nitrate reduction. The alkalinity was higher with these carbon compounds than with the previous ones, due to the fact that, in addition to the alkalinity derived from the nitrogen source, the acid radical of the carbon compound was used as the source of energy and the cation probably left in the medium, forming carbonates, thus resulting in distinctly alkaline reaction. *A. bobili*, *A. reticuli*, *A. reticulus-ruber* and *A. ruber* made a fair growth on the acetic acid, while *A. violaceus-ruber*, *A. griseus*, *A. asteroides*, *A. bovis*, *A. reticulus-ruber* and *A. ruber* produced, with this carbon source, more than scant quantities of nitrites. *A. viridochromogenus*, *A. madurae*, *A. asteroides*, *A. albus*, and *A* 168 made more than a scant growth (*A. asteroides* best) on the lactic acid, while only *A. asteroides* produced more than scant quantities of nitrites in the medium, with this source of carbon. *A. griseus* and *A. viridochromogenus* produced more than a scant growth on malic acid, while *A. violaceus-ruber*, *A. griseus*, *A. asteroides* and *A.* 205 allowed a good accumulation of nitrites in the medium. *A. viridochromogenus*, *A. asteroides*, *A. exfoliatus* and *A.* 168 produced a fair growth on tartaric acid, while only *A. asteroides* allowed a fair accumulation of nitrites with this source of carbon. No organism made more than a scant growth on oxalic acid, while *A. violaceus-ruber*, *A. reticulus-ruber* and *A. hominis* allowed a good to fair nitrite accumulation. This will tend to show that the organic acids form (as sodium salts), with very few exceptions, rather poor sources of carbon for the actinomycetes (succinic acid also gave similar results). Some acids (malic) were offered in the form of ammonium salts and seemed to be used much more readily than the sodium salts. This may be due to the fact that, in the case of the latter, the medium becomes so alkaline that the organisms cannot readily grow further.

UTILIZATION OF NITROGEN COMPOUNDS

The following organisms were used for this study: *A. violaceus-ruber, A. griseus, A. aureus, A. bobili, A. scabies, A. albus, A. viridochromogenus, A. verne, A. ruber, A. bovis. A. asteroides* and *A. reticuli*. Three per cent of glycerin was added (in place of saccharose) to the synthetic solution as the source of carbon; glycerin was used, because, as was shown above, it is readily assimilated by most organisms, and, if it does not allow as abundant a growth of some species as does starch or dextrose, it never allows as poor a growth as the organic acids, arabinose or saccharose; another advantage in the use of glycerin is the fact that the reaction of the medium does not change appreciably on sterilization, as do the media containing dextrose, lactose or maltose. The nitrogen compounds were studied only as sources of nitrogen and not of carbon, which was possible in the presence of a good source of carbon, such as glycerin. These compounds were added to the synthetic solution, the organic compounds 0.5 per cent, the inorganic salts 0.2 per cent. The cultures were grown in duplicates and incubation took place at 25° for 15 and 30 days and, in the case of *A. bovis* and *A. asteroides* for 30 and 60 days. All these studies, as well as the experiments on the utilization of carbon compounds, were carried on in solution; had a solid medium been used, a better growth might have been obtained with the poorer sources of carbon and nitrogen.

Sodium nitrate. Sodium nitrate was used as the only source of nitrogen in the previous investigations on the utilization of carbon compounds. *A. violaceus-ruber, A. bobili, A. griseus, A. scabies, A. verne, A. bovis, A. asteroides* and *A. reticuli* produced only a scant to fair growth on this medium. *A. viridochromogenus* and *A. ruber* produced a fair growth, *A. aureus* and *A. albus* an excellent growth in 30 days. The reaction was changed in nearly all cases to alkaline, only *A. violaceus-ruber* changing it to slightly acid and *A. asteroides* to distinctly acid. Few actinomyces produce a very heavy growth with Na NO$_3$ as a source of nitrogen and glycerin as a source of carbon, many produce a fair to good growth and many a scant growth in solution. When agar is added to the medium, the growth is much heavier.

Sodium nitrite. Those organisms that use well sodium nitrate as a source of nitrogen, will grow readily on sodium nitrite, with very few exceptions, especially if the latter is present in low concentrations (0.02 per cent or less); *A. aureus, A. violaceus-ruber, A. scabies* and *A. viridochromogenus* made a good growth while the other species grew only scantily. *A. violaceus-ruber, A. aureus, A. viridochromogenus, A. scabies, A. verne,* and *A. asteroides* changed the reaction of the medium to acid, while the others did not produce any change in reaction or made the medium faintly alkaline. *A. scabies* and *A. viridochromogenus* produced a soluble brown pigment, *A. griseus* a yellowish and *A. violaceus-ruber* a purple pigment. The amount of growth on NaNO$_2$ is not so abundant as on NaNO$_3$, due to the fact that NaNO$_2$, in too large concentrations, exerts a toxic effect upon the organism. Although, in this experiment,

only 0.2 per cent of NaNO$_2$ was used, it is quite possible that for many organisms, even this concentration is toxic. If the concentration of NaNO$_2$ is reduced too much, it is soon exhausted by the organism. There is no doubt that the organisms that reduce nitrates actively will grow readily with NaNO$_2$ as a source of nitrogen, when present in not too large concentrations; while those species which do not reduce the nitrates appreciably did not make much of a growth on NaNO$_2$, in the concentrations studied. By varying the concentration of the NaNO$_2$ from 5 to 500 mgm. per 100 cc. of solution, it was found that most species grew readily in the low concentrations, using up all the nitrite in a few days; with the increase in concentration of the NaNO$_2$, the toxicity was increased and, at the highest concentration, most species refused to grow at all, while few made some growth. Thus the different species vary in their ability to withstand higher concentrations.

Ammonium salts. Ammonium carbonate and ammonium sulfate offer rather poor sources of nitrogen to the actinomycetes. Only *A. aureus* made a fair growth on the first and an excellent growth on the second in 30–40 days at 25°. *A. ruber* made a scant growth on the carbonate and a fair growth on the sulfate. All the other species studied made none or only a very scant growth on these compounds with glycerin as a source of carbon. With dextrose the growth on the ammonium salts as sources of nitrogen was much more abundant. Only *A. reticuli* made no growth on the sulfate and a scant growth on the carbonate and *A. madurae* grew scantily on the first and made no growth on the second with dextrose as a source of carbon. The only organism that grew well (good) on the sulfate was *A. scabies*, all the others making a scant to fair growth on it. With ammonium carbonate as a source of nitrogen and dextrose as a source of carbon, *A aureus* and *A. violaceus-ruber* made a very good growth, *A. asteroides, A. viridochromogenus, A. verne and A. scabies* made a good growth, and *A. bovis* and *A. asteroides* gave only a fair growth. In the presence of ammonium sulfate, the reaction in all cases became acid, both with glycerin and dextrose (P$_H$ changing from 5.8 to 4.6–4.2 with dextrose). The same thing holds true for the ammonium carbonate (*A. aureus* changing P$_H$ from 6.8 to 4.4), only *A. madurae, A. bovis*, and *A. reticuli* changed the reaction in the presence of the carbonate to slightly alkaline. The limited growth of the organism on the ammonium sulfate medium and to some extent also on the carbonte is no doubt due to the fact that it uses up the cation as the source of nitrogen, leaving the anion in the medium, This results, in a medium rather poorly buffered (only 0.1 per cent K$_2$HPO$_4$), in a distinct change in reaction, so that it soon becomes distinctly acid and below the maximum acidity at which the organisms can grow. It was pointed elsewhere and will also be shown in another connection in this paper that the limiting P$_H$ (on the acid side) for the growth of the actinomycetes is 4.8–5.2. In the case of ammonium sulfate and carbonate media, the reaction was changed, in most instances, to P$_H$ 4.2–4.6, which falls below the maximum acidity that the organisms can tolerate, this leading to a cessation of growth. When the ammonium

is offered in a form where the acid radical is used up as well (ammonium malate), the amount of utilization of this source of nitrogen is greatly increased.

Urea and acetamide. Urea and acetamide allowed only a scant growth of most actinomycetes, with glycerin as a source of carbon. *A. violaceus-ruber, A. aureus* (acetamide only), *A. bobili* (acetamide only), *A. scabies, A. ruber,* and *A. viridochromogenus* (urea only) made a fair to good growth. The reaction was either unchanged or changed very faintly to acid or alkaline. The utilization of urea, as a source of nitrogen, was tested with dextrose as a source of carbon. Most organisms made here also only a faint growth, but *A. violaceus-ruber, A. aureus, A. viridochromogenus* and *A. asteroides* made a very good to excellent growth. The change in reaction was in all cases to alkaline (P_x changed from P_H 7.4 as high as P_H 8.6), except *A. viridochromogenus,* which left the medium slightly acid. The utilization of a definite nitrogen source depends in many cases on the source of carbon. The amides, which form rather poor sources of nitrogen for the group as a whole, with very few exceptions, and are entirely valueless as sources of carbon, will show distinct variation depending on the source of carbon; in the presence of a good source of carbon, some species may make a very good growth; this fact can help a great deal in the separation of the different organisms.

Glycocoll. Glycocoll forms a good to very good source of nitrogen. *A. bovis* and *A. asteroides* were the only two species which did not make any more than a fair growth in 30 days at 25°. An abundant growth was accompanied by a distinct decrease in the amino nitrogen content of the medium, while a slight growth resulted only in a very small decrease. This fact can be used to advantage to measure the utilization of the amino acids by micro-organisms and can be taken, to a certain extent, as an indication of the growth made. *A. aureus, A. viridochromogenus* and *A. reticuli* produced a brown pigment, while the pigment produced by *A. griseus, A. albus,* and *A. bovis* was yellowish, and that of *A. violaceus-ruber* red, in 30 days. The reaction was changed in most instances to alkaline, except *A. violaceus-ruber* (P_x changed from 7.1 to 6.4 in 15 days and 5.6 in 30 days) and *A. asteroides* (P_H from 7.1 to 6.4 in 30 days). Most organisms produced slight quantities of ammonia from glycocoll.

Leucin. Leucin, as well as asparagin and glycocoll, offer good sources of nitrogen for actinomycetes. *A. bobili, A. scabies, A. ruber, A. viridochromogenus* and *A. reticuli* produced soluble brown pigments, that of *A. aureus* and *A. griseus* was yellowish and of *A. violaceus-ruber* red. These same pigments were produced by these organisms on asparagin. The utilization of the nitrogen in leucin, as well as in glycocoll and asparagin, is measured by the decrease in the amino nitrogen content of the medium. Only *A. scabies, A. bovis* and *A. asteroides* produced traces of ammonia in the leucin cultures. The interesting point to be noticed here is the decrease of the hydrogen-ion concentration of the cultures (the reaction becoming always more or less acid, P_H was changed from 7.3 by *A. scabies* to 7.1 in 30 days and *A. violaceus-ruber* to 5.6 in same period, all other species falling between these two).

Asparagin. Only *A. bobili* and *A. asteroides* grew scantily on this source of nitrogen and all the other species made a fair to excellent growth. Ammonia was produced readily by most species in appreciable amounts, so that this compound can be used more readily than the other two for testing the ammonia-producing power of actinomycetes. The reaction was changed in most cases to alkaline, except *A. violaceus-ruber*, *A. aureus*, *A. viridochromogenus* and *A. asteroides* that changed it to acid.

Asparagin, as well as the amino acids, is used readily as a source of carbon and nitrogen. In the absence of any other available source of carbon the organisms grew very readily on asparagin, which served as a source of both carbon and nitrogen.

Fibrin, casein, egg-albumin and peptone. Cultures containing proteins or peptone as sources of nitrogen allow a maximum growth of actinomycetes. Only *A. bobili* and *A. reticuli* produced a scant growth on egg-albumin in 15 days, the growth becoming good in 30 days; otherwise it was chiefly good or very good. Most of the species studied produced a yellow to brown soluble pigment, except *A. violaceus-ruber*, which produced a red or blue pigment depending on the reaction of the culture; *A. verne* produced a brownish pigment only on peptone, *A. albus*, a yellowish pigment on fibrin and casein and *A. asteroides* no pigment at all. The reaction was changed on fibrin, in most cases, to acid, except *A. bovis*, *A. verne*, *A. ruber* and *A. scabies;* on casein to acid, with the same exceptions, including *A. bobili;* on peptone to acid with the same exceptions as on fibrin, also *A. albus;* on egg albumin the reaction of the cultures studied was changed to acid, except *A. griseus,A. ruber* and *A.bobili*, *A. scabies*, *A. verne*, *A. bovis*, and *A. reticuli*, which left the reaction unchanged or made the medium more alkaline. These four organisms, *A. bovis*, *A. verne*, *A. scabies* and *A. ruber* changed the reaction of the cultures containing different proteins or peptone always to alkaline, while other organisms such as *A. violaceus-ruber*, *A. asteroides*, *A. aureus*, *A. viridochromogenus* changed the same media always to acid. The proteins and peptone were hydrolyzed to some extent by the organisms (directly or by means of enzymes) to amino-nitrogen rich compounds, although no general conclusion can be drawn here, since some species affected a greater splitting of one protein and others of another. Ammonia was readily produced by different organisms from the different proteins.

Casein, fibrin, asparagin, and other proteins and amino acids can be used as sources of both carbon and nitrogen. Most species grew very rapidly on these proteins and amino acids, in the absence of any other carbon compounds, deriving from them both their energy and their nitrogen supply. The reaction usually becomes alkaline probably because of the accumulation of ammonia in the medium. The amino-nitrogen content of the media containing the proteins increases considerably and decreases in the media containing the amino acids, which is readily explained by the metabolism of these organisms.

Creatinine. All species tested produced a "fair" to "good" growth in solution on creatinine as the only source of nitrogen. The reaction of the medium

was changed in most cases to faintly acid; *A*. 205 produced a characteristic yellow pigment; *A. violaceus-ruber* a bluish and the few chromogenus species a brownish pigment.   •

Tyrosin. The utilization of tyrosin as a source of nitrogen was tested both on agar and in solution, with saccharose and glycerin as sources of carbon. Most species grew readily on agar, but only *A. scabies* and *A.* 205 produced a soluble brown pigment, indicating that only these two species produced tyrosinase.   Not all the strains of *A. scabies* produced the brown pigment on tyrosin; the strains isolated by the writer did it readily, while those obtained from other sources, particularly when grown for several years on artificial media, seem to have lost that property or produced only a faint brown pigment. When all the species that produced soluble brown pigments on organic media were tested in tyrosin solution (synthetic solution containing glycerin and free from any other sources of nitrogen), *A. scabies* produced a deep brown color, *A. viridochromogenus*, *A. pheochromogenus* and *A.* 205 produced faint brown pigments while *A. violaceus-ruber* produced a soluble blue color.   The growth of all the species was fair to good.

We can thus readily see that the production of a brown pigment on protein media is not a result of the action of tyrosinase alone, since this pigment is formed only by few species from tyrosin, while several other species are able to produce a soluble brown pigment on tyrosin-free media, such as the media containing the other amino acids, inorganic nitrogen salts and proteins free from tyrosin.   The only conclusion that we can make here would be that, as Beijerinck (3) suggested, tyrosinase is a mixture of two enzymes, both of which are produced by *A. scabies* and the other species producing the pigment on tyrosin; the other species producing pigments on other amino acids and proteins are able to produce oxidases which can form brown pigments from organic substances free from tyrosin.   This fact can be further emphasized by the fact that a few species can produce soluble pigment including brown (*A. exfoliatus*) on inorganic media, free from any traces of proteins or amino acids.

Aerial mycelium was formed by most of the species tested on the different nitrogen media, except *A. verne* and *A. bobili*, which did not form any aerial mycelium on all these media; *A. scabies* produced aerial mycelium only on casein and *A. bovis* on casein and egg-albumin.

Milk (table 3).   The milk cultures of the actinomycetes present interesting differences, these depending on the proteolytic powers of the species.   A complete discussion of this subject will be found elsewhere (43).

The chemical changes produced in milk due to the action of actinomycetes can be used in the identification of the different species.   One group of organisms, including *A. griseus*, *A. poolensis*, *A. madurae*, *A. ruber*, and *A.* 206, which coagulated the milk in 3–5 days, soon begin to peptonize the curd, and have it all digested in about 10 days.   The amount of amino-nitrogen and ammonia produced by these organisms is very great, as for example, *A. griseus* converted in 15 days 57.7 per cent, *A. madurae* 59.8 per cent and *A. poolensis*

34.6 per cent of the nitrogen of the milk into amino nitrogen. *A. griseus* converted 23.3, *A. poolensis* 21.8, *A. ruber* 20.1 and *A.* 206, 23.0 per cent of the nitrogen of the milk into ammonia in 40 days. The reaction of the milk was changed by these species to the most alkaline.

The second group of organisms contains those species that coagulate the milk rapidly, but peptonize the curds slowly, so that in most cases the curd is not digested even in 45 days. We would include in this group *A. reticuli*, *A. reticulus-ruber*, *A. rutgersensis*, *A.* 161 and *A.* 145. It would seem that these organisms produce as strong a rennet-like enzyme as the first group of organisms, but a weaker proteolytic enzyme, which therefore accounts for the slow peptonization of the curd. These species are further characterized, in most instances, by the fact that the reaction of the milk is either unchanged as in the case of *A. reticuli* and *A.* 145, or made only faintly alkaline.

Several species, namely *A.* 128, *A. hominis*, *A. viridochromogenus*, *A. diastaticus*, and *A. verne* stand between the two groups, producing a rapid coagulation, while the peptonization is more slow than for group 1 and more rapid than for group 2.

A. fradii, *A. lipmanii*, *A. bovis*, *A. scabies*, *A.* 96, *A. citreus* and *A. luteus* coagulate the milk only in 10–12 days, followed by a rather rapid peptonization. These organisms are characterized by an alkalinity lower than that of group 1 but higher than that of group 2, no doubt due to the fairly rapid digestion of the clot.

A fourth group would comprise those species which do not coagulate the milk, but hydrolyze it without previous coagulation. Here we would include *A. albus*, *A. exfoliatus*, *A. violaceus-ruber*, *A. bobili*, *A. lavendulae*, *A. roseus* and *A. alboflavus*. These are all characterized by a very high alkalinity. It is very possible that the lack of coagulation is not due to the lack of a rennet-like enzyme, but merely to the fact that the digestion of the casein of the milk proceeds rapidly at its early stages, so that coagulation is not observed or is absent. That some of the organisms that coagulate the milk may produce hydrolysis without clotting can also be concluded from the fact that a few cultures (*A. citreus*, *A.* 168 and *A.* 205) produced hydrolysis of the milk in some cases while in other cases the same species produced a clot and then digested it.

Some of these species included in this group were found, on further study, to be able to produce a clot in the milk under certain conditions, depending on the temperature of incubation, amount of inoculum and mother culture. The effect of environmental conditions on the metabolism of actinomycetes is taken up elsewhere (43). Finally we find a few species that seem to have no visible action upon the milk, although some digestion of the milk proteins has taken place; *A. aureus* and *A. asteroides* would belong to this group. The reaction of the milk became in most instances alkaline and in no case acid, although several species produced no change in the reaction of the milk.

The cultures reported above were all incubated at 37°; at this temperature there is hardly any visible growth, although the action on the milk is very definite. At 25° most species readily produce on milk a visible growth accompanied by characteristic reactions, but these reactions are, for many species, not the same as those obtained at 25°. A glance at table 3 reveals this fact. The difference in reaction upon the milk at 25° and 37° is explained by difference in growth, enzyme production and activity of the enzymes at the two different temperatures. So, for example, *A. madurae* and *A. hominis* which grow more readily at the higher temperature, will produce at that temperature a more rapid clot formation followed by the peptonization of the clot; *A. lavendulae*, which grows more readily at the lower temperature, will hydrolyze the milk in a shorter period of time. The changes in reaction, with very few exceptions, run parallel at both temperatures, although for 37° only an approximate change is given while for 25° the exact reaction is reported. The species belonging to the *chromogenus* groups possess a rather weak proteolytic power. All these species, when grown on milk at 25°, produced a surface brown to black ring accompanied by an imperfect clot formation and scant digestion; the amino-nitrogen content of the milk was rather low and the reaction quite alkaline ($++$ to $+++$).

Gelatin (table 4). Fifteen per cent gelatin in distilled water offers a good medium for the study of actinomycetes. All the species, with the exception of *A. asteroides*, liquefied the gelatin in 30-40 days at 18°. But the rapidity of liquefaction and the production of a soluble pigment are characteristic of the species, although these, particularly the rapidity of liquefaction, may change to some extent on continued cultivation upon artificial culture media. The rapidity of liquefaction is also influenced by the temperature of incubation. If the reaction of the gelatin is not adjusted, the culture becomes in most cases more alkaline, with very few exceptions. The gelatin is hydrolyzed, as a result of the growth of the organisms, with an increase in the amino nitrogen content of the medium. On the addition of 1 per cent starch to the gelatin, the latter is usually split to a smaller extent, while the hydrogen-ion concentration of the culture is usually greater (more acid) than in the absence of starch. Nearly all the species are able to utilize gelatin as a source of both carbon and nitrogen. In the presence of an available carbohydrate, the gelatin is used only as a source of nitrogen and therefore is hydrolyzed to a lesser extent.

Glucose broth (table 4). The ordinary glucose broth offers a very good medium for the cultivation of most species, although the growth itself is not characteristic. The growth is usually accompanied by an increase in the amino nitrogen content of the medium, a change in the hydrogen-ion concentration, depending on the initial reaction, and by the production of a soluble pigment.

THE REACTION OF THE MEDIUM

A detailed study on the influence of reaction upon the growth of actinomy-cetes and the change of reaction of culture media has been published elsewhere (43). The reaction was determined as the hydrogen-ion concentration meas-ured by the hydrogen electrode or phenol-sulphone-phthalein series of indi-cators. The limiting hydrogen ion concentrations are about P_H 5.0 and P_H 9.0 for the group as a whole, although several species ($A. reticulus-ruber$) are able to grow at higher hydrogen-ion concentrations (P_H 4.6–4.8); the opti-mum reaction is at P_H 7.0–7.8. In most cases, if the initial reaction of the medium is too acid, it is made, in the course of the growth of the organisms, more alkaline, tending to bring the reaction toward the optimum; if the reac-tion of the medium is too alkaline, it is made more acid, also tending toward the optimum. So we find that gelatin having a P_H equal to 6.2 and milk of a similar reaction are changed in the majority of cases to a more alkaline reac-tion; while glucose broth which is originally alkaline (P_H 7.9) is changed, in the majority of cases, to a more acid condition. But this is not the rule, since several species are able to leave even distinctly acid media unchanged or make them even slightly more acid.

The change of reaction of the medium depends on the nitrogen source and the amount of buffer in the medium. When ammonium sulfate is present and the medium is rather poorly buffered (only 0.1 per cent K_2HPO_4) the medium soon turns distinctly acid (P_H 4.4–4.8), stopping any further growth of the organisms. The same holds true for other salts of ammonia, where the acid radical is not used up at the same time. In the case of ammonium salts of organic acids, such as ammonium malate, where the ammonium is used as a source of nitrogen and the malate as the only source of carbon, the reaction always changes to alkaline due to the fact that the malate is used up more readily as a source of energy than the ammonia as a source of nitrogen, the energy requirement of the organisms being greater than that of nitrogen.

Ammonium carbonate is used also much more readily than the sulfate, because the hydrogen-ion concentration is increased, in most cases, to a smaller extent. This is readily explained, since carbonic acid is much less ionized than sulfuric acid (or hydrochloric), hence we would expect that the ammonium salts of a weakly dissociated acid, even if the acid radical is not used by the organisms, would offer better sources of nitrogen than the salts of strong acids, because the latter will make the reaction of the medium distinctly more acid, unless a large amount of buffer is present.

When sodium nitrate is present as the only source of nitrogen the reaction is changed, in most cases, with nearly all sources of carbon to more alkaline. With sodium nitrite, the reaction is changed, in most cases, to more acid. This is explained (43) by the assumption that most species reduce the nitrate to nitrite and use the nitrogen in that form; in the process of reduction the hydrogen tension of the medium would be decreased, this tending to a lower hydrogen-ion concentration or a more alkaline reaction.

In the presence of organic nitrogenous substances, the medium may become either alkaline or acid depending on the initial reaction of the medium and the organisms. When the medium, richly buffered with proteins, such as milk, gelatin, glucose broth, etc., is acid to start with, it will become in most instances, more alkaline; when it is alkaline to start with, it tends, in many instances, to become more acid. The presence of certain nitrogenous substances, such as leucin, tyrosin, creatinine, tend to leave the medium always more acid, to a greater or less extent, at least with certain carbohydrates. Some species (*A. scabies*) tend to turn the medium, in the presence of proteins or peptones always more alkaline, other species (*A. asteroides, A.* 145) usually make the reaction of the same media more acid. It should be noted here that any figure in the paper enclosed in parentheses refers to the last word only.

INFLUENCE OF TEMPERATURE

The influence of temperature upon distribution and growth of microörganisms is an important factor in the study of a particular group. It was first of all important to find out the maximum and minimum temperatures for the growth of actinomycetes, and then the temperatures which destroy the species studied. Glucose broth was used for the cultivation of the species. Most organisms were found to grow readily at temperatures ranging from 15° to 37°, the higher temperature favoring a more rapid growth, so that a certain species would require 10–15 days to give the same amount of growth at 15–18° as it would give at 37° in 24–48 hours. Particularly is this true of *A. madurae, A. hominis* and *A. bovis*, which grew readily at the higher temperature and very slowly at the lower temperature. At 40°, *A. bovis, A. scabies, A. ruber* and *A. violaceus-ruber* made a scant and *A. griseus* a fair growth. At 45° none of these five organisms made any growth at all.

A. griseus, A. bovis, A. scabies, A. ruber and *A. violaceus-ruber* were grown on glucose broth at 25° for 8 days. The cultures were then kept for 1 hour at 70°, 75°, 80° and 90°; and then tested for sterility by inoculating from the glucose broth upon glucose agar and nutrient agar. All the 5 cultures survived 70° and 75° for 1 hour. *A. bovis* was killed and the other 4 species survived a temperature of 80° for 1 hour. Only *A. griseus* survived a temperature of 90° for 1 hour. The mycelium is destroyed at lower temperatures than the spores, so that, at 90°, *A. griseus* survived only in the spore form, while the mycelium was destroyed; the mycelium of *A. scabies* seems to be destroyed at 80°, although the spores survive that temperature and are destroyed at 90°.

A temperature of 75° is sufficient to destroy the rennetic enzyme of these organisms. *A. griseus* milk cultures added to fresh sterilized milk and kept for 24–48 hours at 75° gave no action upon the milk. The temperature has an effect not only upon the rapidity of clotting and subsequent peptonization of the milk by the cultures but also upon the mode of action. Twenty tubes of

sterile milk were inoculated with *A. griseus* and 20 tubes with *A. exfoliatus*, and 10 tubes of each culture placed at 37° and at 25°. At 37°, all the *A. griseus* cultures formed a clot in 5–6 days and digestion began in 6 days and was nearly completed in 10 days. Of the 10 cultures of *A. exfoliatus*, 3 clotted in 9 days and all were clotted in 13 days; the digestion of the milk in these cultures began in 13 days and was not completed in 30 days. At 25°, of the 10 *A. griseus* milk cultures, 2 clotted in 6 days and all clotted in 10 days; peptonization of the clot began soon after this was formed and was completed in 14–15 days. *A. exfoliatus* produced no clot on the 10 tubes at 25° but hydrolysis of the milk became apparent in 25 days and most of the tubes cleared up in 30–35 days.

PRODUCTION OF ENZYMES

With the exception of the rennetic and proteolytic enzymes, as was shown elsewhere, no attempts were made to isolate the enzymes of the different actinomycetes. Most species produce readily diastatic, rennetic and proteolytic enzymes. Some produce a cellase, as demonstrated by the cellulose plate method; invertase is produced only by a few species.

The production of tyrosinase has been pointed out in another connection in this paper. The hemolysis of the blood is no doubt accomplished by an hemolysin-like enzyme, which is produced by the most active proteolytic species. The proteolytic enzyme liquefies and splits gelatin readily; casein is readily hydrolyzed; the fact that coagulated blood serum is liquefied while coagulated egg-albumin, with very few exceptions, is not liquefied by many species would seem to indicate that the former is attacked by the proteolytic enzyme, while the latter is not. When milk cultures of a few species were filtered and filtrates precipitated by means of alcohol, preparations were obtained which possessed very active rennetic and proteolytic properties.

SUMMARY OF COMPARATIVE CULTURAL DATA

1. All the actinomycetes can be grown readily in artificial culture media, both synthetic and organic.

2. Some species show distinctive cultural differences when grown in artificial media soon after isolation from natural substrata and after they were, kept in culture for a number of years. This is true only of some species and not of all of them.

3. Arabinose is not assimilated by most species. Dextrose, maltose, lactose, mannite, glycerin and starch are readily assimilated by most species to a greater or less extent, very few cultures producing only a scant growth on these sources of carbon; saccharose is assimilated readily by some species, although nearly all organisms made some growth on this source of carbon, particularly in agar media. Cellulose is readily assimilated only by some species. Inulin is readily assimilated by most species.

4. $NaNO_3$ is assimilated by all species in the presence of a favorable source of carbon. $NaNO_2$ is readily assimilated by most species but only in very low concentrations. Ammonium salts are assimilated readily only by very few species, but in the presence of favorable sources of carbon, such as dextrose, they are assimilated to some extent by many species. Urea and acetamide are assimilated only to a very small extent, and only few organisms make a very good growth on these sources of nitrogen. The proteins and amino acids form the best sources of nitrogen for most actinomycetes; creatinine is readily used, but not to such an extent as the proteins.

5. Most actinomycetes grow readily on milk. Very few produce any visible surface growth at 37°, but produce a good growth at 25°; the milk is usually clotted and peptonized; few species hydrolyze the milk, without any previous clotting and some produce no visible action upon the milk; the hydrolysis and the lack of visible action upon the milk by some species is not absolute, since some of these cultures may at other times, particularly at favorable temperatures, clot and peptonize the milk. The reaction of the milk is usually changed to alkaline; no species renders the milk acid, while a few do not change the reaction of the milk.

6. Gelatin in distilled water forms a good medium for nearly all species. The gelatin is liquefied by nearly all species with different rapidity, with or without the production of a soluble brown pigment in the liquefied and often unliquefied portion.

7. Some species are characterized by the production of a brown pigment in gelatin as well as in other media containing proteins or amino acids; this pigment is not due to the action of tyrosinase only, since on tyrosin only a few species produced the pigment, while the latter was also produced in media not containing tyrosin.

8. Blood serum, blood agar and whole egg form good media for the cultivation of most species; liquefaction of the coagulated serum, hemolysis of the blood and the production of a soluble purple to black pigment on all the three media is characteristic of several species.

9. Potato and carrot can be readily used for the cultivation of most species, some of which produce a characteristic growth.

10. The optimum range of temperature for the growth of most actinomycetes is 30–37°. The lower the temperature, the slower is the growth of the organisms. Above 37°, the growth rapidly diminishes and at 45° only one organism made a scant growth. A temperature of 80° for 1 hour is sufficient to kill most species; only one form (*A. griseus*) survived that temperature and was killed when kept at 90° for 1 hour. The character of the growth and biochemical activities may vary somewhat with the different temperatures.

11. Most of the actinomycetes are very active proteolytically, splitting the proteins to amino acids and ammonia. In this respect they differ somewhat from some molds and some bacteria which produce a great deal of ammonia as a final product. The protein hydrolysis may stop at the amino acid (poly-

peptide) stage and very little of it may be reduced to ammonia, particularly in a short period of incubation; when the period of incubation is prolonged (30–60 days) very large quantities of ammonia accumulate.

12. Most actinomycetes reduce nitrates to nitrites, depending on the source of carbon; some species do that only to a very limited extent and with one or two sources of carbon, while others reduce nitrates readily with practically all sources of carbon. The nitrite production seems to be a step of the utilization of the nitrate nitrogen for at least some organisms; this may explain the absence of nitrites in certain cases, since the nitrite is assimilated by the organism as soon as formed. The organisms that reduce nitrates readily can use nitrites as the only source of nitrogen, when present in small amounts.

13. The following enzymes are produced by most actinomyces species: rennet like, proteolytic and diastatic enzymes. Inulase and invertase are produced only by certain species, and tyrosinase by very few. The hemolysis of blood and the utilization of cellulose may also be brought about by the particular enzymes.

14. In summarizing the cultural and biochemical studies, the following media can be recommended for a starting point in studying and identifying the different species:

(a) Synthetic agar No. 1 and glucose agar (Krainsky's) as standard synthetic media; temperature of incubation 22–25°, period of incubation 7–15 days.

(b) Gelatin, 15 per cent, in distilled water; reaction unadjusted; temperature of incubation 16–18°; period of incubation 30 days.

(c) Skimmed milk; temperature of incubation 25° and 37°; observations to be made daily.

(d) Potato plugs at 25° for 7–15 days.

(e) Starch agar at 25° for 10–15 days, test for diastatic power.

(f) Nutrient agar, 25° for 7–15 days (optional).

(g) Tyrosin solution, 25° for 15–20 days (optional).

(h) Loeffler's blood serum, 37° for 7–15 days (optional).

VARIABILITY

The species of Actinomyces are among the most variable groups of micro-organisms; in the study of actinomycetes, as well as bacteria, not only the influence of the medium and previous substrata on which the particular species were grown, but the great variability, within rather wide limits, of the species or rather "species group" itself should be considered. When the same culture grown on different artificial culture media for a period of time, and often even for one short generation, is studied morphologically and physiologically, notable variations are observed, so that the untrained observers would be apt to take these as representing different forms. Often several strains of the same "species group," when compared on the same culture medium, might be taken

culturally and often morphologically as distinctly different species. Quite often one culture, on the same medium, under the same conditions of temperature and length of incubation, will show distinctive differences. ·

How shall then the limits of variability of a particular species be defined? The morphological characters alone would be far from sufficient. First of all, the substratum mycelium is not very characteristic and is quite uniform for many species; only the aerial mycelium shows the distinctive morphological characters. But the aerial mycelium may often not be produced at all by several species; others may not form any aerial mycelium on certain media, while forming it on others; still other species may lose the ability to form the aerial mycelium altogether on continued cultivation on artificial culture media. Then, the same culture may show slight differences in morphology on the same medium at different periods, or at one period on the different media. Even more striking than the morphological are the physiological variations. These depend chiefly on the substratum of the mother culture, temperature and length of incubation, amount and kind of inoculum (vegetative or aerial mycelium or spores). For example, a certain culture (A. griseus) may at one time clot the milk at 37° in 2 days and then peptonize it (dissolve the clot) in 5–6 days; at another time, the same culture, under the same conditions, will clot the milk only in 5–6 days, then peptonize it in 12–15 days; while at a third time, some tubes may not show any clot at all, and the milk is hydrolyzed (cleared up without any previous clot). Another culture (A. exfoliatus) may show persistently an ability to hydrolyze milk without forming any clot at 37°, then it may form a very good clot, followed by peptonization; when incubated on the same lot of milk from the same mother culture, it may show a clot followed by peptonization at 37°, while at 25° only hydrolysis may take place, at another time a clot is formed also at 25°. The rapidity of liquefaction of gelatin is also subject to a great variation. Still more intense is the variability, when such factors as the amount of split products obtained from a certain protein or carbohydrate are considered. All these factors come to show that ordinary bacteriological methods will not hold when applied to this group of microörganisms. The amount of variation cannot be fixed so easily and if it were done, it would be very arbitrary.

On closer intensive study of this group of organisms, we find that the variations, although often very striking, are mostly of a quantitative rather than qualitative nature. It was suggested above that these organisms should be classified in groups rather than as individual species. The characters, particularly the cultural and biochemical, should be studied repeatedly, under different conditions, then the data obtained compared, due allowances being made for the variability, and the culture will then be properly located. This idea was kept continuously in mind in the above studies; many of the data show rather a range, obtained from repeated cultures, than a single observation. If attempts were made to describe all the details of the cultures and to make new

species based on some variations from others, the 40 or so species described above could have been easily increased to several thousand, the task becoming more and more laborious and almost unnecessary. And, since it became almost impossible to carry on in continuous culture numerous strains, all of them were studied on several media, which allowed a better differentiation of their morphological and cultural differences. The great majority of the strains were then discarded and only the strains representing the "species groups" left. Repeated study and collective comparison of data have helped to bring forth some of the distinctive characters of these "species groups." An example of forming one can be found in the strains of *A. griseus* and *A.* 218 which are as different from one another as any two strains of one "species group" (with the exception of the "*A. scabies*" group); descriptions of both of them are given so as to indicate the extent of variability allowed by the writer.

SAPROPHYTES AND PARASITES

Most of the species studied in this paper are saprophytic in nature. *A. scabies* is pathogenic to plants, while *A. madurae*, *A. hominis*, *A. bovis* and *A. asteroides* are supposedly animal pathogens. There is absolutely no difference between the pathogenic and saprophytic forms. Parasitism of a few species is not sufficient reason to have them separated in a different group and all attempts in that respect are only superficial in nature. The methods outlined for the study of this group are applied just as readily to both saprophytes and parasites. The only distinctive difference is found in the optimum incubation temperature: that of the animal pathogens is higher than that of saprophytes and plant pathogens, which we would naturally expect.

MORPHOLOGICAL STUDIES

The reasons have been presented above, why the morphological studies here will have to be only very limited, in view of the very detailed studies of Drechsler (13). Attention will be called here to only a few facts, chiefly the variability of the morphological characters depending on the culture medium. It is we1 to describe these organisms on one definite medium, but when several media lare used, great variations are obtained. It is then very important to have t he one medium used of exact chemical composition so that the work may be readily repeated. A few plates are given in the end of this paper to point out this variability and also to indicate some characteristic morphological features of the different species.

Three distinct morphological types are recognized:

a. Whirl formation, as represented by only two species: *A. reticuli* and *A. reticulus-ruber*. This feature is very characteristic and cannot fail to help recognize the group, but it was observed only on the synthetic (saccharose) agar, and even on this medium, the second species has shown only limited whirl formation (plate 3).

b. Those species that do not form any spirals in the aerial mycelium, forming only straight branching hyphae. Also in this respect, we find great variability on the different media (plate 4).

c. Those species that form spirals. These comprise the majority of the actinomycetes, and distinctive differences are found in type and method of formation of spirals as well as in the variation on the culture medium. Some form wide, open spirals, while others form narrow spirals of a corkscrew type (plates 1 and 2). The division into dextrorose and sinistrorose types of spirals has already been pointed out by Drechsler (13).

KEY TO THE IDENTIFICATION OF THE SPECIES

(Based chiefly on biochemical characters)

A. Formation of a soluble pigment on all media containing protein substances:
    I. Pigment deep brown (chromogenus types):
        1. A brown pigment is produced on tyrosin agar:
            (a) Pigment dark brown; white to cream-colored growth on synthetic media; soluble brown pigment on synthetic media containing arabinose, dextrose or lactose................................*A. scabies*
            (b) Pigment faint brown; sulfur-yellow soluble pigment on creatinine solution; aerial mycelium on glucose agar is ocher to reddish ocher colored.
                                                         Actinomyces 205
        2. Growth and aerial mycelium on synthetic agar green to dark-green; soluble brown pigment on synthetic media with most carbohydrates.
                                                A. viridochromogenus
        3. Deep brown to black pigment on synthetic agar:
            (a) Weakly growing organisms; orange-red growth on potato plug; no visible aerial mycelium on synthetic agar...........*A. purpeochromogenus*
            (b) Vigorously growing organisms; brown to black growth on potato plug; abundant cottony aerial mycelium on synthetic agar.
                                             A. pheochromogenus
        4. Usually no action on milk (37°), accompanied by the darkening of the milk; mouse-gray aerial mycelium on synthetic agar; ammonium salts used readily with different sources of carbon...........................*A. aureus*
        5. Brown pigment never produced on synthetic media:
            (a) Aerial mycelium on synthetic media has lavender shade ...*A. lavendulae*
            (b) Aerial mycelium on synthetic agar is abundant, of a water green color.
                                        Actinomyces 218
            (c) Whirl formation in aerial mycelium on synthetic agar:
                (a') Growth colorless and aerial mycelium white.........*A. reticuli*
                (b') Growth pink, aerial mycelium rose colored; nitrate reduction very abundant; fewer whirls.............*A. reticulus-ruber*
            (d) Growth on synthetic agar sulfur-yellow, with yellow aerial myceliun; barnacle-like, greenish-yellow growth on potato plug......*A. flavus*
            (e) Growth on synthetic agar red colored, aerial mycelium abundant, orange colored; aerial hyphae usually do not form spirals...........*A. ruber*

    II. Soluble pigment on organic media faint brown, golden, yellow or blue:
        1. Pigment blue, not always definite; soluble red turning blue pigment on synthetic agar.......................................*A. violaceus-ruber*

2. Pigment at first green on organic media and synthetic agar, property lost on
   continued cultivation, becoming brown on synthetic agar; aerial mycelium
   not produced on most media................................*A. verne*
3. Soluble pigment at first brown, property lost entirely on continued cultiva-
   tion; growth and aerial mycelium on synthetic agar abundant, white.
   A. albus
4. Soluble pigment yellowish green; growth on synthetic agar penetrating into
   the medium is pink...................................*A. californicus*
5. Soluble pigment on organic media (gelatin and glucose broth) golden; sulfur-
   yellow growth on synthetic agar with yellow soluble pigment (last property
   lost on continued cultivation)...................................*A.* 168
6. Brown pigment produced only on certain protein media (usually gelatin and
   glucose broth, not nutrient agar):
   (a) Growth on synthetic agar red to pink; no differentiated aerial mycelium
       or only scant white...................................*A. bobili*
   (b) Growth on synthetic agar colorless; aerial mycelium thin, rose-colored.
       A. roseus
   (c) Growth on carrot and potato rapidly spreading, enveloping the whole
       plug and destroying it rapidly, plug becoming colored deeply brown.
       A. 96
   (d) Red (vinaceous) soluble pigment on synthetic agar, often turning red-
       brown; white aerial mycelium...........................*A.* 161
   (e) Reaction of organic media always becoming acid; weak proteolytic
       action; very coarse aerial hyphae, without any spiral production.
       Actinomyces 145

B. No soluble pigment produced on gelatin or other protein media:
   I. Species strongly proteolytic; gelatin liquefied rapidly, milk clotted and peptonized
   rapidly.
   1. Brown soluble pigment on synthetic agar; diastatic action very strong.
      A. diastaticus
   2. Rapid liquefaction of coagulated blood serum, strong hemolysis of blood (37°):
      (a) Very poor utilization of glycerin as a source of energy; aerial mycelium
          on synthetic agar has a tea-green tinge.................*A. griseus*
      (b) Colorless growth on synthetic agar with white aerial mycelium; pink
          growth on glucose agar with scant aerial mycelium; growth slow at
          25°, rapid at 37°; pathogenic to man..................*A. madurae*
      (c) Yellowish growth on the 3 synthetic agars; aerial mycelium on synthetic
          agar has an olive-green tinge (very similar to that of *A. griseus*);
          yellowish to orange, growth turning brown on potato plug; olive-col-
          ored growth on carrot; pathogenic to man..............*A. hominis*
   3. Yellowish green growth on starch plate with pinkish aerial mycelium; citron-
      yellow growth on synthetic agar...........................*A. citreus*
   4. Greenish-yellow growth on synthetic agar, gray powdery aerial mycelium,
      greenish-yellow soluble pigment.............................*A.* 128
   5. Colorless growth on synthetic agar, white to grayish aerial mycelium, no spiral
      formation; thin reddish-brown growth on potato plug (purplish zone on
      plug); faint yellow pigment may develop on gelatin, blood and egg-media.
      A. poolensis
   6. Buff colored growth on glucose agar, violet-gray aerial mycelium; yellow
      growth on synthetic agar with light drab aerial mycelium; rapid destruction
      of potato plug..*Actinomyces* 206
   7. Proteolytic action somewhat weaker than previous members of group B I,
      although much stronger than the species included in B II.

(a) Yellow growth on most synthetic and organic media, often turning black, sulfur-yellow aerial mycelium, which usually develops only on continued cultivation; pathogenic to animals............*A. bovis*

(b) Very scant colorless growth with scant white aerial mycelium on synthetic agar and on synthetic media containing NaNO₃ as a source of nitrogen; abundant brown growth with white aerial mycelium and soluble brown pigment on glucose agar; growth on potato plug greenish turning black.................................*Actinomyces* 104

II. Proteolytic action weak:
   1. Soluble pigment produced on synthetic agar:
      (a) Pigment blue or blue-black......................*A. violaceus-caesari*
      (b) Pigment brown to almost black on all synthetic media with NaNO₃ as a source of nitrogen...............................*A. exfoliatus*
   2. No soluble pigment on synthetic agar, although growth is colored:
      (a) Growth turning black, diastatic action very strong:
         (a') Growth on synthetic agar scant with abundant spirals in aerial mycelium, no invertase production...........*A. rutgersensis*
         (b') No spirals on synthetic agar, characteristic green colored growth on protein-glycerin media......................*A. lipmanii*
         (c') None or scant aerial mycelium on all media; growth abundant on synthetic agar (invertase positive); none or scant growth on blood agar and egg-media......................*A. halstedii*
      (b) Growth orange colored on most synthetic and organic media:
         (a') Aerial mycelium pink............................*A. fradii*
         (b') White aerial mycelium may or may not be produced; proteolytic and diastatic action lacking almost entirely (this is the only species which does not liquefy gelatin and has no visible effect upon milk at 25° and 37°)....................*A. asteroides*
      (c) Growth yellowish on synthetic and glucose agars: pinkish cinnamon-colored on calcium malate agar; no growth on blood serum and egg media; none or only very scant and late aerial mycelium on most media..*A. albo̧flavus*
      (d) Growth on synthetic media rose to red colored, aerial mycelium white, no visible action on milk..........................*A. albosporeus*

The writer is well aware of the several criticisms to which this key could be subjected. First of all, pigment production, which is never an absolutely reliable factor and which is subject to variations, is made use of in the major and minor subdivisions. Second, the major divisions of part B are based on quantitative differences in proteolytic action, which also is not always reliable. With all these criticisms in mind, the present key was decided on, since it presents a brief summary of the more important biochemical features of the different species which will help to separate them. It will no doubt be of help to those who will attempt to locate a certain culture. The key should of course be used only as a preliminary step in the identification; for further details, one should refer to the complete description. At least those media should be used which are recommended in the last paragraph of the summary of the cultural characters.

Attention is here called to the fact that the most of the cultural studies reported above were repeated several times at different laboratories by the writer and often also by others (associated with the writer, notably Mr. Curtis and Mr. Joffe of the New Jersey Agricultural Experiment Station).

TABLE 1

*The utilization of different carbon compounds by actinomycetes**

ORGANISM	Arabinose	Dextrose	Saccharose	Lactose	Maltose	Starch	Inulin	Cellulose†		Mannite	Glycerin	Acetate	Lactate	‡Malate	Tastrate
								I	II						
A. violaceus-ruber	1	5	2–3	5	4	5	2	0	3	3	3	1	—	1 (3)	0
A. violaceus-caesari	1	3	2	1	2	3	—	2	3	1	0	1	1	1	1
A. aureus	0	4	1	4	—	2	—	0	0	2	3	1	1	1 (4)	0
A. scabies	4	2	2	4	2	4	—	1	—	3	3	1	1	1	1
A. viridochromogenus	3	4	2	3	4	3	2	0	3	3	2	1	2	2	1
Actinomycetes 205	0	1	1	2	4	3	2	2	0	1	1	1	0	1	1
A. albus	2	3	2	3	3	3	—	2	0	3	3	1	2	0	1
A. exfoliatus	4	4	5	4	3	4	2	0	1	3	3	1	1	1	1
A. griseus	3	4	1	3	4	4	1	0	0	3	2	1	0	3	1
A. albosporeus	0	3	2	2	1	3	—	1	0	4	1	1	1	1	1
A. lipmanii	0	3	3	3	—	1	—	3	—	3	3	—	—	—	—
A. diastaticus	4	4	1	4	3	3	2	2	0	4	4	0	1	1 (2)	1
A. bobili	0	2	1	3	2	3	—	1	0	0	3	2	1	1	0
A. poolensis	0	1	1	2	1	3	—	3	0	1	4	0	0	1	1
A. fradii	3	4	1	3	2	4	2	0	0	3	3	1	1	1	0
Actinomyces 128	0	2	1	2	3	5	—	3	0	1	1	1	1	0 (3)	1
A. roseus	1	3	2	0	—	4	—	0	—	1	2	—	—	—	—
A. verne	0	1	1	2	2	3	—	1	1	3	1	—	1	0 (1)	1
A. reticulus-ruber	0	4	1	2	4	1	2	1	1	0	3	2	1	1	1
Actinomyces 168	0	5	2	4	3	2	—	2	1	5	4	1	2	1	1
A. ruber	1	3	2	3	4	4	3	2	2	3	3	2	0	1	1
A. asteroides	0	4	3	0	3	4	—	0	1	0	2	1	2	1 (2)	—
Actinomyces 104	0	1	1	1	2	0	—	1	1	1	1	1	1	1	1

* NaNO₃ (2 gm. per liter) used as a source of nitrogen. All carbon compounds were used in a 3 per cent concentration.

† I, designates filter paper; II, reprecipitated cellulose.

‡ Figures enclosed in parentheses designate the utilization of the malate in the form of the ammonium salt, as the only source of nitrogen and carbon.

ORGANISM	Fibrin	Casein	Egg-Albumin	Peptone	Creatinine	Glycocoll	Leucin	Tyrosin	Asparagin	Urea g	Urea d	Acetamide	NaNO₂	NaNO₃	(NH₄)₂SO₄ g	(NH₄)₂SO₄ d	(NH₄)₂CO₃ g	(NH₄)₂CO₃ d
A. violaceus-ruber	3‡	3	2	3	3	3	3	2	3	1	5	2	1	3	0	1	0	4
	3	4	2	4	—	4	3	—	4	2	—	1	1	3	0	—	0	—
A. griseus	4	5	4	5	—	4	3	—	3	1	—	1	1	1	0	—	0	—
	4	5	4	5	—	3	3	—	3	1	—	1	2	1	1	—	0	—
A. aureus	3	4	4	4	3	5	4	3	4	1	4	2	3	3	1	2	1	4
	4	5	5	5	—	5	5	—	4	1	—	2	5	3	5	—	2	—
A. bobili	2	3	1	2	—	2	2	—	1	1	1	1	1	0	0	—	0	2
	3	4	3	4	—	3	—	—	1	1	—	2	—	0	0	—	0	2
A. scabies	3	3	2	3	3	2	2	2	2	1	2	1	1	1	0	—	0	3
	5	4	3	4	—	3	3	—	2	2	—	2	2	3	0	—	0	—
A. albus	3	2	3	2	—	3	3	—	2	1	—	1	1	1	0	—	0	—
	3	3	4	4	—	4	3	—	—	1	—	1	5	1	1	—	0	—
A. viridochromogenus	3	4	3	4	3	3	3	3	2	3	4	1	2	1	0	1	0	3
	3	5	—	4	—	5	3	—	2	—	—	1	2	3	0	⊣	0	—
A. verne	2	2	2	3	—	3	3	—	2	1	1	1	1	1	0	0	0	3
	2	3	2	3	—	3	3	—	3	1	—	—	—	1	0	—	0	—
A. bovis	2	3	3	2	—	2	2	—	2	1	2	1	1	1	0	1-2	0	2
	3	4	4	2	—	2	2	—	3	1	—	1	1	—	0	0	0	—
A. asteroides	1	1	3	2	—	2	2	—	1	1	4	1	1	0	0	2	0	3
	1	1	3	2	—	2	2	—	1	1	—	1	1	—	0	—	0	—
A. reticuli	4	—	1	4	—	3	3	—	3	0	1	0	1	1	0	0	0	1
	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Actinomyces 205	3	3	2	3	3	3	3	3	3	0	—	1	2	1	2	—	0	—
	5	3	4	4	—	5	4	—	5	3	—	2	2	1	2	—	1	—

* Organic nitrogenous substances were used in concentrations of 0.5 per cent (creatinine and tyrosin only 0.1 per cent); nitrite, nitrate and ammonium salts 0.2 per cent.

Three per cent of glycerin was used as a source of energy for all nitrogen compounds unless otherwise designated.

† Column marked "g" designates glycerin and "d" designates dextrose (3 per cent) as source of energy.

‡ 0, designates no growth; 1, faint; 2, fair; 3, good; 4, very good; 5, excellent; —, not tested. The figures in the upper row were obtained on incubating the cultures for 15–18 days (*A. bovis* and *A. asteroides* for 30 days) at 25°; those in the lower row were obtained for the cultures incubated 30–35 days (*A. bovis* and *A. asteroides* 60 days).

TABLE 3

The growth of actinomycetes on milk

ORGANISM	INCUBATION AT 37°—DAYS								INCUBATION AT 25°—DAYS							
	Coagulation	Peptonization Start	Peptonization End*	Peptonization Rapid-ity	Hydrolysis	No action	Browning	Reaction	Coagulation	Peptonization Start	Peptonization End*	Peptonization Rapid-ity	Hydrolysis	No action	Browning	Reaction
A. aboflavus	—	—	—	—	10-12	—	—	+	—	—	—	—	20+	—	—	7.5
A. albosporeus	—	—	—	—	—	+	—	0	—	—	—	—	20	—	—	7.7
A. albus	—	—	—	—	20	—	—	+	—	—	—	—	20-25	—	—	8.0
A. asteroides	—	—	—	—	—	++	—	0	—	—	—	—	—	++	—	6.2
A. aureus	—	—	—	+	15-18	+	+	0	Soft cloth	—	—	+	15	—	+	7.3
A. bobili	10-12	12	40	+	—	—	—	++	10-12	10-12	20+	—	—	—	—	8.0
A. bovis	15	15	30+	+	—	+	—	++	5-6	5-6	15-20	—	—	—	—	8.2
A. californicus	9-10	9-10	20	+	—	—	—	++	—	—	—	—	15+	—	—	8.0
A. citreus	—	—	—	—	—	—	+	++ +	5	—	—	+++	—	—	+	7.0
A. chromogenous 205	5-7	7	18-25	++	—	—	—	++	5-6	5-6	20	++	—	—	—	8.0
A. diastaticus	6-7	7-8	50+	+	—	—	—	+++	4	4	20	++	—	—	+	5.4
A. 161	—	—	—	++	—	—	—	+++	5-6	5-6	50+	+++	—	—	—	6.4
A. exfoliatus	3-6	3-6	15-30	++	8-10	—	—	+	—	—	—	—	—	—	—	
A. 128	10-12	10-12	—	+++	—	—	—	+++	5	5	20	+++	15	—	—	7.8
A. flavus	10-20	10-20	20	+++	+	—	—	+++	—	—	—	+++	20+	—	+	7.8
A. fradii	4-5	5	10	+++	—	—	—	+++	10	10	20	++++	—	—	—	5.4
A. 96	—	—	—	++++	—	—	—	++	3-5	3-5	20	—	—	—	—	7.8
A. griseus	—	—	—	—	15-20	—	—	+++	3-5	—	—	—	20	—	—	8.3
A. 218	10	10	50+	+	—	—	—	+++	—	—	—	+	—	—	+	8.0
A. halstedii	5-6	5-6	20	+++	—	—	—	+++	8-10	8-10	30+	—	10	—	+	7.6
A. hominis	—	—	—	—	—	—	—	+++	—	—	—	—	—	—	—	8.2
A. lavendulae	8-9	9-10	30	++	20-30	—	—	++	Soft	—	—	—	3-4 / 10-12	—	—	7.8

							Yellow pigment				pH
A. 168............	5-8	6-8	20	++	−	−		−	−	20	7.9
A. madurae........	3-4	3-4	10-30	+++	−	−		−	−	−	8.2
A. pheochromogenus....	20	20	50+	+++	−	−		+	−	−	6.6
A. poolensis.......	4-5	4-5	9-10	+++	−	−		−	−	−	7.6
A. purpeochromogenus...	10	10-12	50+	+++	−	−		+	−	−	6.6
A. reticuli........	4-6	6	50+	+++	−	−		−	+	−	7.5
A. reticulus-ruber...	4-6	6	50+	+++	−	−		−	−	10	7.0
A. roseus.........	−	−	−	−	10-15	−		−	−	−	8.2
A. ruber..........	3-4	3-4	7-10	+++	−	−		−	−	−	8.0
A. rulgersensis....	4-6	5-6	50+	+	−	−		+	−	−	7.4
A. scabies........	5-10	5-10	10-30	+++	−	−		+	−	−	6.6
A. verne..........	4-5	5	18	+++	−	−		−	+	−	7.8
A. violaceus-caesari..	10-12	10-12	50+	+	−	−		−	+	−	6.6
A. violaceus-ruber...	−	−	−	−	12-15	−	Pinkish color	−	−	20	8.2
A. viridochromogenus..	5-6	6	12	+++	−	−		+	−	−	7.1
A. 104............	−	−	−	−	−	−		−	−	20	8.2
A. 145............	6-7	7	50+	++	−	−		−	+	−	6.4
A. 206............	4-5	4-5	10-12	++++	−	−		−	−	20	6.3

* A figure with sign + designates that the peptonization was not yet completed in that period of time.

TABLE 6

Comparative cultures of actinomycetes on gelatin and liquid media

ORGANISM	GELATIN 15 DEGREES C.		GLUCOSE BROTH		SYNTHETIC SOLUTION		GLYCERIN SYNTHETIC SOLUTION	
	Pigment	Liquefaction*	Growth	Pigment	Growth	Nitrites	Growth	Nitrites
A. albosporeus	None	Rapid	Colonies	None	Colonies	3	Flakes	T
A. albuporeus	None	Rapid	Ring	None	Flakes	2	Flakes and colonies	2
A. albus	Brownish	Medium	Ring	None	Colonies	1-3	Flakes	1
A. asteroides	None	None	Pellicle	None	Flakes	2-4	Flakes	2
A. aureus	Brown	Medium	Ring	Brown	Flakes and colonies	0-1	Flakes	1
A. bobili	Brown	Rapid	Flakes	Brown	Colonies	3	Colonies and flakes	T
A. lawit	None	Medium	Ring	None	0	4	Flakes	4
A. californicus	None	Medium	Pellicle	None	Flakes	5	Pellicle	O
A. citreus	None	Medium	Ring	None	Flakes	0	Flakes and pellicle	T
A. chromogenus 205	Brown	Slow	Ring	Brown	Flakes	1	Pellicle and flakes	T
A. diastaticus	None	Rapid	Colonies	None	Flakes	—	Pellicle	T
A. 161	None	Slow	Ring or pellicle	Brown	Ring	0-2	Pellicle and flakes	T
A. erfoliatus	None	Slow	Colonies	None	Colonies	3	Colonies	T
A. 128	None	Rapid	Pellicle	None	Colonies	0-1	Flakes	1
A. flavus	Brown	Rapid	Colonies	None	Colonies	1	Colonies and pellicle	T
A. fradii	None	Rapid	Ring	None	Colonies	3	Pellicle and flakes	1
A. 90	Yellow	Medium	Ring	Brown	Flakes	0	0	2
A. griseus	None	Rapid	Pellicle	Brown	Flakes	1	Flakes	T
A. 218	Brown	Medium	Ring	Brown	Flakes	2	Pellicle	3

A. halstedii	None	Rapid	Colonies	None	Colonies	0-2	Flakes	T
A. homissis	None	Rapid	Ring	None	0	0-3	Pellicle	3
A. lavendulae	Brown	Medium	Flakes	None	Colonies	0-1	Pellicle	T
A. lipmanii	None	Rapid	Ring	None	Flakes	3	Flakes	1
A. 168	Golden	Rapid	Pellicle	Golden	Pellicle	4	Pellicle	0
A. madurae	None	Rapid	Colonies	None	0	0-1	Flakes	1
A. phaeochromogenus	Brown	Slow	Ring	Brown	0	0-2	Pellicle	4
A. poolensis	None	Rapid	Ring	None	0	0-1	Flakes	2
A. purpeochromogenus	Brown	Slow	Flakes	None	Flakes	0	Flakes	0
A. reticuli	Brown	Medium	Colonies	Brown	Flakes	2		
A. reticulus-ruber	Brown	Rapid	Flakes	Brown	Colonies	4	Colonies	1
A. roseus	Brown	Slow	Flakes	Brown	Flakes	3		
A. ruber	Brown	Medium	Ring and colonies	None	Flakes	0-3	Colonies	1
A. rutgersensis	Brown	Medium	Colonies	None	Flakes	3	Pellicle	4
A. scabies	Brown	Slow	Colonies	Brown	Colonies	0-1	Colonies and flakes	0
A. verne	None	Rapid	Flakes	None	Colonies	3	Flakes	2
A. violaceus-caesari	None	Slow	Flakes	None	Flakes	0-2	Colonies	T
A. violaceus-ruber	None	Slow	Ring	Blue	Colonies	5	Colonies	3
A. viridochromogenus	Brown	Slow	Ring	Brown	Flakes and colonies	1-2	Pellicle and flakes	0
A. 104	None	Rapid	Pellicle	None	Flakes		Flakes	1
A. 145	Brown	Medium	Pellicle	Brown	0		0	2

* Liquefaction is designated as slow, when, on plates or tubes, only sinking of growth or narrow liquefied zone is obtained at 18° in 12 days; rapid, when half or more of tube (10 cc.) is liquefied or the liquefied zone on plate is 1 cm. or more wide in 15-20 days at 18°; medium, when a moderate amount of liquefaction is obtained.

† Growth is designated as "colonies," when colonies are floating through medium, on surface of liquid or bottom of tube; "ring," when a surface growth forms a ring in contact with glass of tube; "pellicle" indicating a surface pellicle; "flakes" when a mass of flakes is present through medium or on bottom (usually) of tube.

‡ Relative amount of nitrites obtained at 25° in 15 days from NaNO₃ with different sources of carbon.

TABLE 5

Summary of comparative cultures of actinomyces on agar media

ORGANISM	SYNTHETIC AGAR			CALCIUM MALATE AGAR		GLUCOSE AGAR		NUTRIENT AGAR			STARCH AGAR	
	Spirals	Growth*	Aerial mycelium*†	Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Soluble pigment	Growth†	Aerial mycelium
A. violaceus-ruber...	Numerous	Red-blue	Gray	Red	Gray	Brick-red	Gray	Colorless-red	Gray	None-reddish brown	Pink	Gray
A. violaceus-caesari..	Numerous	Gray-bluish	White	Blue	White	Red	White	Gray	None	None	Bluish	Gray
A. viridochromogenus	Numerous	Dark-green	White-green	Dark-green	White-bluish	Gray-black	White-bluish	Gray	White	Brown	Yellowish	Greenish
A. scabies...	None or few	Gray	White	Yellowish	Gray	Gray	None	White	None	Brown	White	White
A. purpeochromogenus	None or few	Brown	Black	Black	None	Brown	Dark-brown	Gray-Crown	None	Brown	Brown	None
A. phaeochromogenus	Numerous	Brown	White	Brownish	White	Brown	White	Gray	None	Brown	Brown	White
Actinomyces 205...	Numerous	White	Gray	White	Gray	Brown	White-gray	Brown	White	Brown	White	Gray
A. aureus...	Numerous	White	Dark-green	White	Brown	Light-orange	Light-drab	Gray	None	Brown	White	Buff
A. lavendulae...	Numerous	White	Lavender	White	Lavender	Yellowish	White-lavender	Gray	None	Brown	White	Lavender
A. bobili...	None or few	Red	None-scant white	Cinna-mon	None	Red	None	Gray-brownish	None	None	Pink	Scant white
A. roseus...	Numerous	White	Vinaceous	White	Rose	White	Pink	White-yellowish	None	None	White	White

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Species													
A. diastaticus	Few or none	Gray	Drab	Brown	Gray	Yellowish	None	White	White	White	None	White	None
A. rutgersensis	Numerous	Brown	Gray	White	Gray	Brownish	White	White	None	Brown	None	Gray	Gray
A. lipmansii	None	Brown	Gray	Brown	Gray	Yellow	None	Yellow	None	None	None	White-dark	None
A. griseus	None or few	White	Greenish	Green-yellow	Greenish	White	White	White	Green	White	None	White	Gray
A. 218	None or few	White	Greenish	White	Greenish	Yellowish	Buff	Brown	White	Brown	Brown	White	White
A. californicus	Numerous	Pink	Gray	White	Gray	Pink	Gray	White	White	White	None	Pink	Gray
A. albus	None	Gray	White	Gray	Gray	Gray	Gray	White	None	None	None	White	None
A. fradii	None	White-orange	Pink	Orange	Pink	Buff	Pink	Orange	None	None	None	White	Pink
A. exfoliatus	None or few	Brown	White	White	White	Brownish	White	White	None	None	None	Brownish	Gray
A. reticuli	None (whirls)	Yellowish	White	White	Yellow	Brownish	Yellow	Brownish	None	Brown	Brown	Brownish	Lavender
A. reticulus-ruber	None or few (whirls)	Pink	Rose to pink	Red	Rose-pink	Red	Pink	Red	None	Brown	Brown	Pinkish	Lavender
A. citreus	Few	Yellow	Yellow	Yellow	Gray	Yellow	White	White	None	None	None	Yellow	Pinkish
A. alboflavus	None	Yellowish	White-none	Pinkish	White (late)	Yellow	None	White	None	None	None	Yellowish	None
A. verne	None	Brownish	None	Avellaneous	Scant white	Gray	None	Gray	None	None	None	Brownish	None
A. albosporeus	None	Pink	White	Rose	White	Red	White	White	None	None	None	Reddish	None
A. halstedii	Few	Gray-brown	Gray	Dark	Gray	Dark-brown	None	White	None	None	None	Brown-white	None
A. flavus	None	Yellow	None	Yellow	White	Yellow	Scant white	Gray	None	Brownish	Brownish	White	White
A. poolensis	None	White	White	White	Gray	Brown	None	Yellowish	None	None	None	White	White

TABLE 5—Concluded

ORGANISM	SYNTHETIC AGAR			CALCIUM MALATE AGAR		GLUCOSE AGAR		NUTRIENT AGAR			STARCH AGAR	
	Spirals	Growth*	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Growth	Aerial mycelium	Soluble pigment	Growth	Aerial mycelium
A. ruber.............	None	Red	Orange	Orange	Yellow	Red	White-pinkish	Olive green	Gray	Brown	Red	Pink
A. 96................	None	White	Gray	Brownish	Gray	White to dark	Gray	Brownish	White	Brown	Brown	Gray
A. 128...............	None	Yellow	Gray	Yellow	Gray	Yellow	Gray	Yellow	Gray	None	Greenish-yellow	Gray
A. 161...............	Numerous	White-yellow	White	White-pink	White	White to brown	White	White	White	None	White	White
A. 168...............	Numerous	Yellow	Gray	White	Gray	White	Gray	White	White	None	White	Gray
A. 206...............	None	Yellow	Gray	Buff	Violet-gray	Buff	Violet-gray	White	None	None	Yellowish	White
A. madurae..........	None or few	White	White	White	Gray	Pinkish	White	White	White	None	White	None
A. hominis...........	None or few	Yellowish	Greenish	Yellowish	Greenish	Yellowish	White	Yellowish	White	None	White	None
A. bovis.............	Few	Yellow	Yellow	Brownish	None	Yellowish-dark	Yellow	Brown	Yellow	None	Yellow	None
A. asteroides........	None	Orange	None	Orange	White	Orange	None	Yellow	White	None	Orange	White
A. 104...............	Numerous	White	Gray	Brownish	None	White	White	White	None	None	White	Gray
A. 145...............	None	White	Gray	White	None	White	Gray	White	Gray	None	White	Gray

* Only color of growth is reported under the column of growth.
† Only color is reported. For detailed description of color of growth and aerial mycelium see complete records.

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TABLE 6

Summary of biochemical activities of actinomycetes

ORGANISM	INVERTASE PRODUC-TION	DIASTATIC ACTION		PROTE-OLYTIC ACTION	CHANGE IN REACTION
		Tube*	Plate†		
A. alboflavus	+	3	...	2–3	Variable
A. albosporeus	+	24	2–4	1–3	Little change
A. albus	−	14	3–4	1–3	Variable
A. asteroides	−	0	0	1	Acid
A. aureus	+	14	2	1	Variable
A. bobili	+	16	3–4	3–4	Unchanged
A. bovis	−	2	...	2	Alkaline
A. californicus	+	..	2–3	2	...
A. chromogenus 205	+	14	3	1–2	Variable
A. citreus	+	2	...	3	Alkaline
A. diastaticus	−	31	5	2–3	Alkaline
A. 161	+	..	3–4	3	Alkaline
A. exfoliatus	+	6	2–4	1–2	Variable
A. 128	−	16	1–3	3–4	Alkaline
A. flavus	+	15	2–3	2–3	Acid
A. fradii	−	17	3–4	3	Alkaline
A. 96	−	2	...	3	Alkaline
A. griseus	−	23	3–4	5	Alkaline
A. 218	−	3	...	3–4	Alkaline
A. halstedii	+	4	...	1–2	Little change
A. hominis	−	3	...	3–4	Variable
A. lavendulae	−	3	...	1–2	Usually acid
A. lipmanii	+	24	4	3	Alkaline
A. 168	−	34	3–4	2–3	Alkaline
A. madurae	−	3	...	3–5	Alkaline
A. pheochromogenus		2	...	1–2	Alkaline
A. poolensis	−	5	2	3–5	Alkaline
A. purpeochromogenus	None−traces	1	...	1–2	Alkaline
A. reticuli	+	..	1–2	1–4	Acid
A. reticulus-ruber	+	12	1–2	1–2	Variable (acid)
A. roseus	−	3	...	3	Alkaline
A. ruber	+	14	2–3	2–3	Variable
A. rutgersensis	−	22	5	3–4	Alkaline
A. scabies	+	0	0–1‡	1–2	Alkaline
A. verne	+	14	4	4	Little change
A. violaceus-caesari	−	15	...	2	Alkaline
A. violaceus-ruber	+	12	...	3	Alkaline
A. viridochromogenus	−	2	12	2	Variable
A. 104	−	8	1–3	2	Variable
A. 145		2	...	1–2	Acid

* Tube = Difference in the height of starch in the control tube and the tube in which the organism was grown, in millimeters.

† Plate = Width of clear zone around the colony, in millimeters. Period of incubation in both instances 12–15 days at 25°C. . . . designates "not tested."

‡ The starch is hydrolyzed chiefly to the dextrin stage.

Comparative cultures of actinomyces upon different media

ORGANISM	BLOOD AGAR Growth	BLOOD AGAR Soluble pigment	BLOOD SERUM Hemolysis	BLOOD SERUM Growth	BLOOD SERUM Soluble pigment	BLOOD SERUM Liquefaction	EGG-MEDIA Growth	EGG-MEDIA Aerial mycelium	EGG-MEDIA Soluble pigment	POTATO PLUG Growth	POTATO PLUG Aerial mycelium	POTATO PLUG Color	CARROT PLUG Growth	CARROT PLUG Aerial mycelium	CARROT PLUG Color
A. alboflavus	—	—	—	None	—	—	None	—	—	White	White	White	White	White	None
A. albosporeus	—	—	—	Pink	None	0	Gray	None	None	Gray	None	None	White	None	None
A. albus	Greenish	None	0	White	None	0	White	White	Purplish	Red-brown	None	None	Pink	Green	None
A. asteroides	Brown	None	0	White	None	0	Yellow	White	None	Yellow	None	None	Orange	White	Brown
A. aureus	Brown	Dark zone	0	Gray	Dark zone	0	Brown	None	Purple	Brown	Gray	Black	None	—	—
A. bovis	Brownish	None	2	Gray	Yellow	3	White	White	None	Yellow	Yellow	Brown	White	Yellow	Dark
A. bobili	—	—	—	Gray	Brown	0	Brown	None	Purple	Yellow-red	Scant white	Black	White	None	None
A. californicus	Gray	None	0	White	Brownish	1	White	Buff	Purplish	Brownish	White-buff	Brown	Gray	—	—
A. chromogenus 205	—	—	.	Brown	Dark zone	0	Gray	Gray	Black zone	Black	None	Black	Gray	White	Brown
A. citreus	Gray	None	1	White	None	0	Red	White	Black	Red	Pink	None	Red	None	None
A. diastaticus	Brown	Brownish	2	Gray	None	1	Yellow	None	Purple	Brownish	None	Brown	Brown	Gray	Brown
A. 161	—	—	—	Gray	None	0	Yellow	White	None	Yellow	Gray	Black	Yellow	Purple	Brown
A. exfoliatus	—	—	—	White	None	0	Orange	White	None	Orange	White	None	Orange	White	None
A. 128	Green	White	4	Gray	White	4	White	White	None	Black	Green	Brown	Gray	Orange	Brown
A. flavus	—	—	—	White	Brown	0	White	None	None	White	White	Black	Green	None	None
A. fradii	Red	None	0	Orange	None	0	White	None	None	White	White	Purple	White	None	None
A. 96	—	—	—	None	—	—	Red	Rose	Dark zone	Green	Red	Black	Brown	None	None
A. griseus	Greenish	None	5	Gray	None	5	White	None	None	Yellowish	Greenish	Brown	None	Gray	None
A. 218	—	—	—	Gray	Brown	1	White	Buff	Purplish	Brownish	White-buff	Brown	Gray	—	—
A. kalstadii	—	—	—	None	—	—	Gray	None	None	Gray	None	None	White	None	None
A. hominis	Gray	None	3	White	None	4	Yellow	None	None	Yellow	White	Brown	Olive	Green	None

Species															
A. lavendulae	—	—	—	Gray	Brown	0	White	None	None	White-yellow	None	Black	Brown	White	None
A. lipmanii	Gray	None	0	White	None	2	White	—	Pink	White	Gray	Purple	Gray	Gray	None
A. 168	Brownish	White	1	Orange	None	1	White	White	None	White	White	Purple	Brown	White	None
A. madurae	Brown	None	5	Yellowish	None	5	Yellow	White		Yellow	Gray	Brown	White	None	None
A. phaeochromogenus	Brown	None-dark	0	Brown	Brown	1	Brown	White	Brown	Brown	White	Black	Brown	White	Black
A. poolensis	Greenish	None-brown	0	Gray	None	1	Yellow	White	Purple	Green-olive	White	Dark	Yellow	None	None
A. purpeochromogenus	—	—	—	None	—	—	Gray	None	None	Orange	None	None	None	—	—
A. reticuli	—	—	—	Gray	None	0	Yellow	None	None	Gray	None	None	White	None	None
A. reticulus-ruber	—	—	—	Gray	Brown	0	Gray	Gray	Purple	Gray	Gray	Black	Yellow	None	None
A. roseus	—	—	—	Gray	None	0	Yellow	None	Purple	Brownish	None	Brown	Brown	None	None
A. ruber	Green	Dark gray	2	Yellow	Brown	0	Brown	None	Brown	Reddish-brown	None	Purple	Brown	Yellow	None
A. rutgersensis	—	—	—	Gray	None	0	Gray	White	Purple	Gray-brown	White	None	White	None	None
A. scabies	Gray	Dark brown	0	Brownish	Brown	0	Brown	None	Black zone	Black	None	None	None	—	—
A. verne	—	—	—	White	None	2	None	—	—	White	White	White	White	White	None
A. violaceus-caesari	—	—	—	Gray	None	0	Gray	None	None	Yellowish-brown	None	None	None	—	—
A. violaceus-ruber	Red	Faint brown	3	Red	Red	0	Brown	White	Blue	Brownish	White-gray	Blue	Brown	Gray	Pink
A. viridochromogenus	Brown	Dark brown	0	Brownish	Dark brown	0	Brown	White	Dark brown	Gray-brown	White	Black	Gray	White	Brown
A. 104	—	—	—	Gray	None	1	White	None	None	Green	None	Black	Brown	None	Brown
A. 145	—	—	—	White	None	0	Brown	None	Purple	Gray	White	Black	White	None	Brown
A. 206	—	—	—	White	None	3	White	White	Pink	White	White	Brown	Brown	White	Brown

* Hemolysis at 37°: 1 = very narrow clear zone in 15 days at 37°; 5 = clear wide zone (2 mm. and more) in 10–15 days; 2, 3, 4 fall between, as relative amounts of hemolysis.

† 0 = no liquefaction at 37°: 1 = growth sinking into serum in 15 days at 37°; 5 = nearly all the slant liquefied in 10–15 days; 2, 3, 4 designate the relative amounts of liquefaction.

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PLATE 1

FIG. 1. *A. violaceus-ruber* grown on dextrose agar.
FIG. 2. *A. violaceus-caesari* grown on dextrose agar.
FIG. 3. *A. violaceus-caesari* grown on calcium malate agar.
FIG. 4. *A. pheochromogenus* grown on dextrose agar.
FIG. 5. *A pheochromogenus* grown on calcium malate agar.
FIG. 6. *A. purpeochromiogenus* grown on starch agar.
FIG. 7. *A. aureus* grown on dextrose agar.
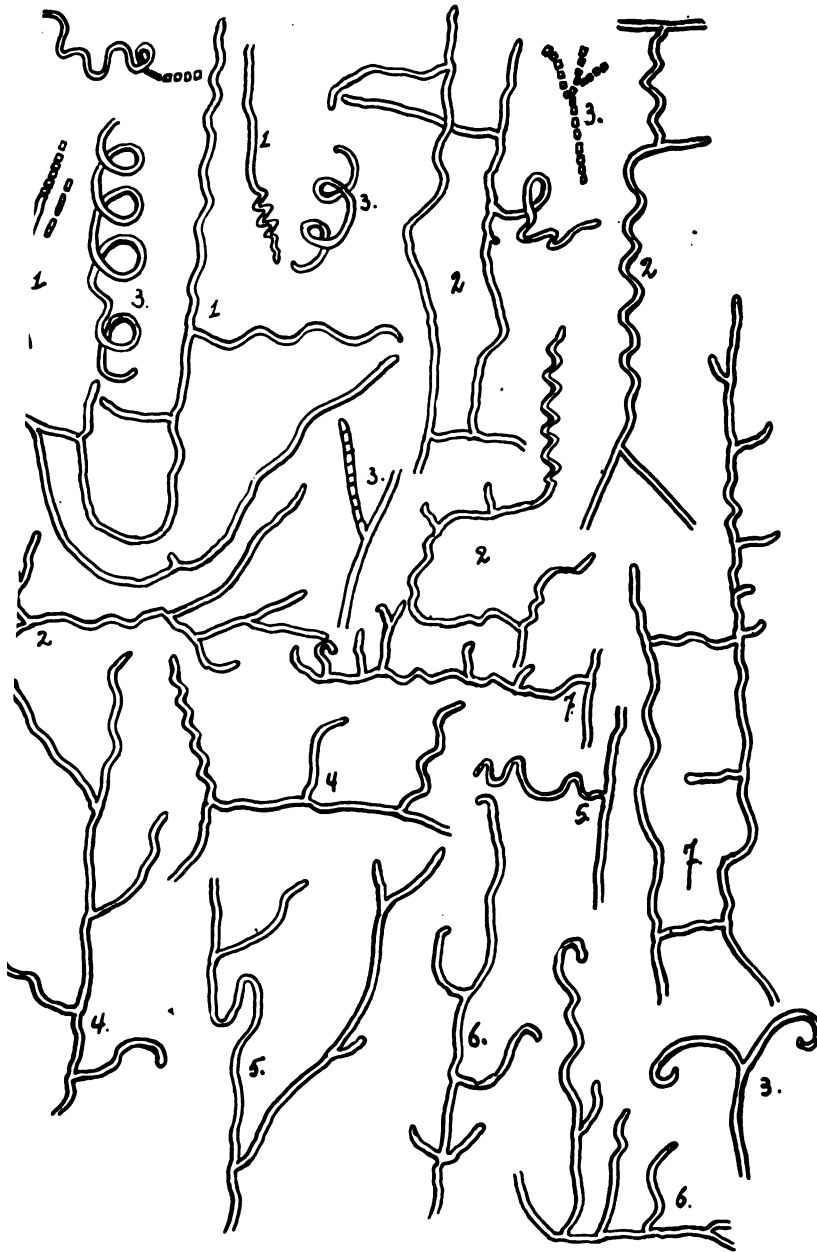
PLATE 2

Fig. 1. *A. alboflavus* grown on calcium malate agar.

Fig. 2–3. *A. citreus* grown on dextrose agar.

Fig. 4. *A. exfoliatus* grown on dextrose agar.

Fig. 5. *A. californicus* grown on starch agar.

Fig. 6. *A. bobili* grown on starch agar.

Fig. 7. *A. ruber* grown on calcium malate agar.

Fig. 8. *Actinomyces* 218 grown on calcium malate agar.

Fig. 9. *A. bovis* grown on dextrose agar.

SELMAN A. WAKSMAN
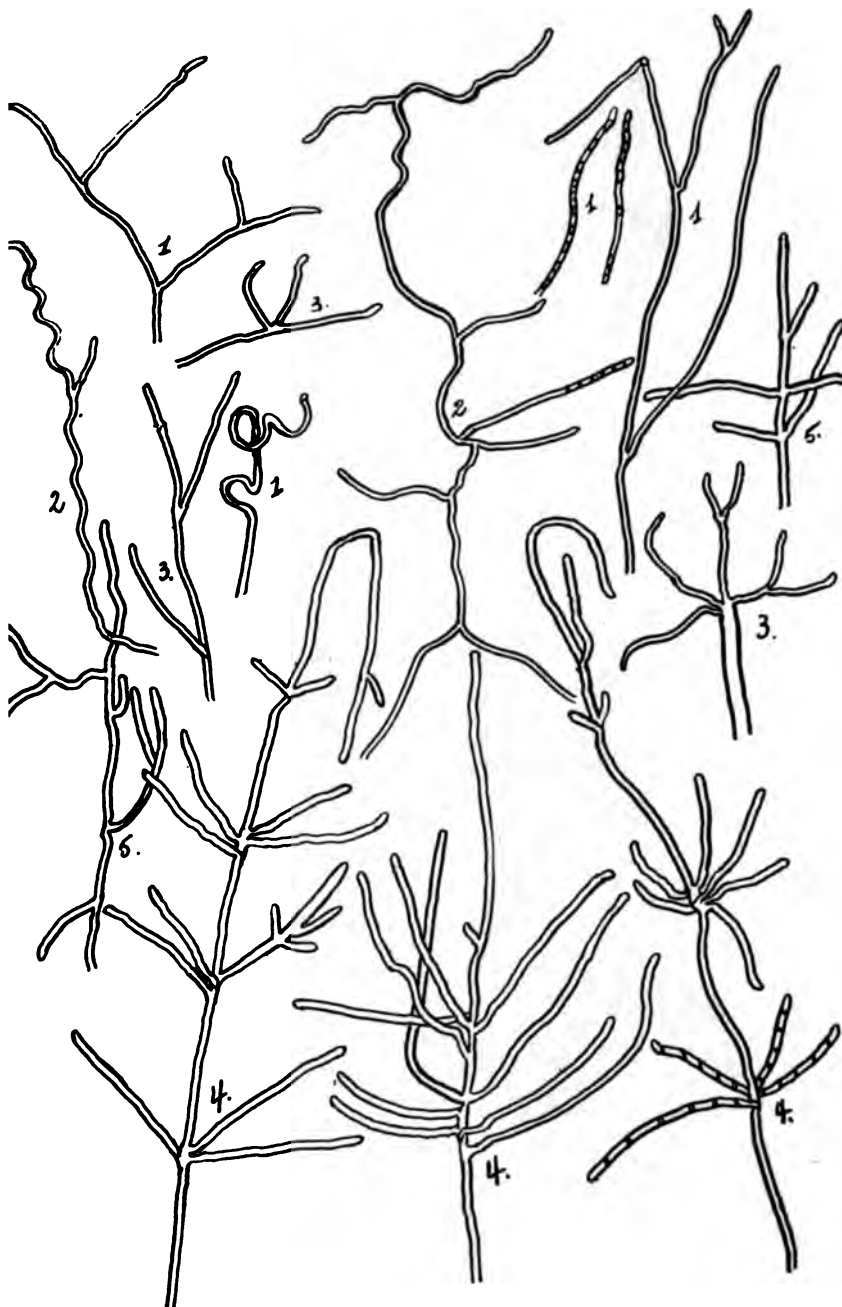
PLATE 2

PLATE 4

FIG. 1. *A. flavus* grown on starch agar.
FIG. 2. *A. flavus* grown on calcium-malate agar.
FIG. 3. *A. roseus* grown on starch agar.
FIG. 4. *A. roseus* grown on dextrose agar.
FIG. 5. *A verne* grown on starch agar.
FIG. 6. *A. lavendulae* grown on dextrose agar.
FIG. 7. *A. flavovirens* grown on calcium malate agar.
FIG. 8. *Actinomyces* 145 grown on dextrose agar.
FIG. 9. *Actinomyces* 145 grown on calcium malate agar.
FIG. 10. *A. aureus* grown on glycerin synthetic agar.

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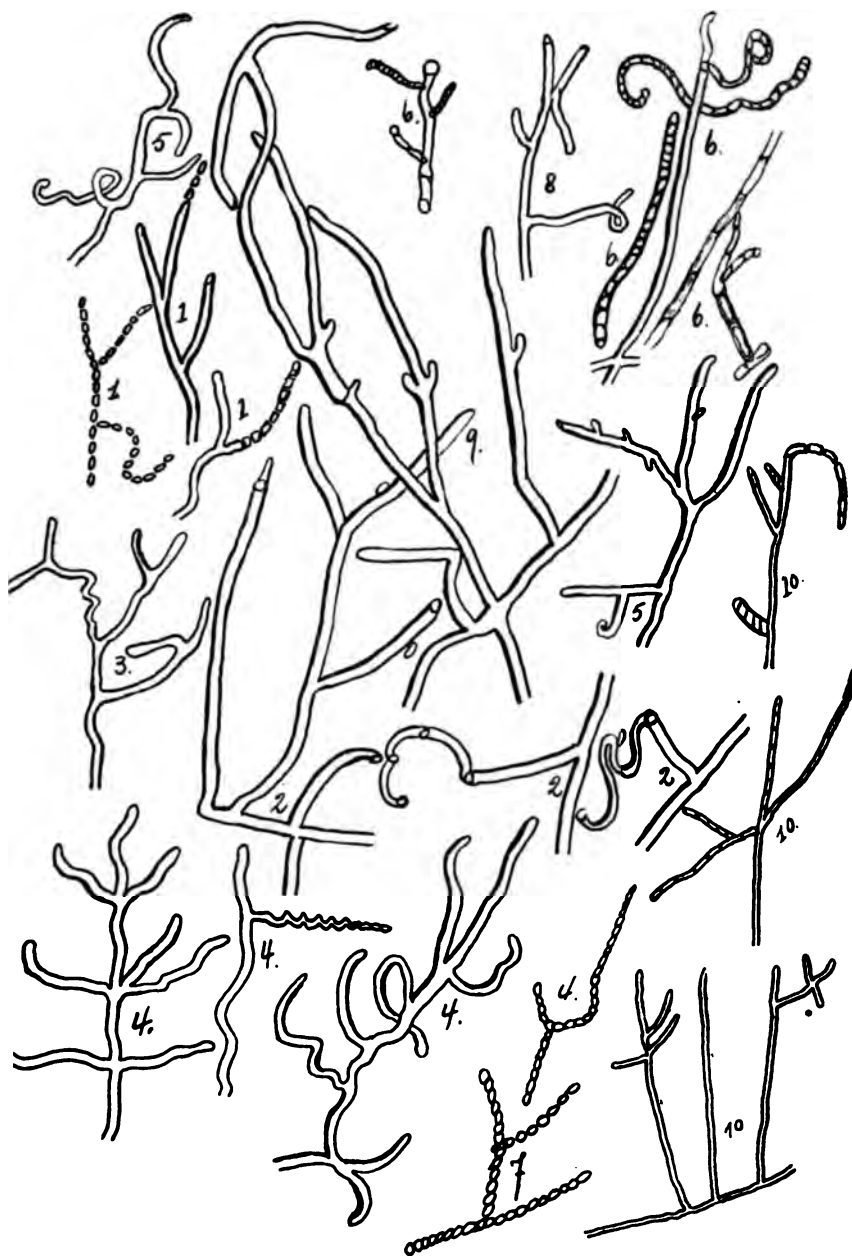
HYDROGEN-ION CONCENTRATION OF PLANT JUICES

I. The Accurate Determination of the Hydrogen-ion Concentration of Plant Juices by Means of the Hydrogen Electrode[1]

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INTRODUCTION

The hydrogen electrode has but recently been employed for the measurements of the hydrogen-ion concentration in plant juices. This method has been used for this purpose by Haas (7), Kappen (10), Hempel (9), and Truog and Meacham (22). The type of apparatus used by these workers gives values of sufficient accuracy for work in which small differences are not significant. The simple type of gas chain is also to be preferred in the electrometric titration method for total acidity in plant juices as used by Haas (8).

The hydrogen electrode was studied critically by Loomis and Acree (11) and applied by them (12) to accurate measurements in organic reactions. Clark (2) has adapted the method for accurate measurements in the case of bacteriological media. Because of the great importance of acidity[2] in plant juices it is desirable to adapt the method to the accurate measurement of this acidity. That the acidity may exert marked influences upon the activities of the plant is evident from the work of Reed (17, 19) and Bunzell (1). These investigators found that a slight acidity inhibits the action of oxidases which are now held to be universally distributed (18) in plants and play an essential rôle in plant respiration. According to Bunzell (1) the concentrations of acidity which completely inhibit the oxidase action for each type of plant material fall within a rather narrow range.

That enzymes are affected markedly by acidity is noted further in the observation of Michaelis (16) that the natural acidity of body fluids containing an enzyme is, as a rule, the optimum acidity for the activity of that

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[2] Acidity and hydrogen-ion concentration are used synonymously in this paper.

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enzyme. Sherman, Thomas and Baldwin (21) have found that amylases representative of the starch-splitting enzymes of the higher animals, higher plants and of fungi, respectively, have an optimum acidity for their greatest activity. Kappen (10) believes that in plants, as in animals, slight changes in acidity influence the activity of the enzymes which are present. In this way the important life processes of the plant are affected. Crocker (4) has suggested that changes in acidity may be important in the regulation of

TABLE 1

Representative measurements showing possible accuracy of the apparatus and method when buffer solutions and plant juices are used

SOLUTION	ELECTRODE VESSEL	TIME			POTENTIOMETER READINGS IN VOLTS		TEMPERATURE	P_H VALUE
		H$_2$ started	Shaking began	Readings taken	Readings	Average of first readings		
Buffer solution of KH$_2$PO$_4$ +NaOH	A	11:45	11:57	12:01	0.68739	0.68738	31.5°	5.801
				12:05	0.68743			
	B	11:45	11:57	12:02	0.68738			
				12:06	0.68736			
Buffer solution of KH$_2$PO$_4$ +NaOH	A	12:35	12:51	12:55	0.68834	0.68837	32.3°	5.809
				12:58	0.68844			
	B	12:35	12:51	12:56	0.68841			
				1:01	0.68852			
Juice of medium red clover	A	3:47	4:00	4:10	0.70977	0.70973	25.0°	6.276
				4:13	0.70973			
	B	3:47	4:00	4:12	0.70969			
				4:15	0.70972			
Juice of soybean seedlings	A	2:15	2:27	2:31	0.69931	0.69928	31.7°	5.965
				2:35	0.69928			
	B	2:15	2:27	2:33	0.69926			
				2:38	0.69925			

transpiration. This suggestion is made from the work of MacDougal (14) and MacDougal and Spoehr (15) on the effects of acids and bases on imbibition of water by plant tissues and plant gels. It seems possible that certain conditions may change the acidity of the plant juice sufficiently to produce an acidity which is unfavorable for the plant.

In a study of the factors affecting the acidity of plant juices the possible effect of soil acidity is one of considerable importance. Measurements made by Truog and Meacham (22) bear upon this relation These results indicate

that the acidity of the plant juice may often be increased by an acid reaction of the soil. Since differences caused by such factors may in many cases be quite small and yet be of great importance, it seemed desirable to adapt the hydrogen electrode method of measurement to the determination of plant acidity so as to get much more accurate values of plant acidity than have previously been obtained. A procedure and apparatus which will give accurate values of acidity with one kind of material may not always work similarly with other material. The Clark and Lubs (3) procedure and apparatus gives accurate values with bacteriological media but do not always give accurate values with plant juices. It is probable that substances present at least in some plant juices are the cause of considerable difficulty (6).

THE APPARATUS

The apparatus used in the present investigations is similar in its essentials to the chain described by Clark and Lubs (2) which embodies the use of the Clark electrode vessel, devised for shaking, a constant-temperature air-bath, electrolytically prepared hydrogen and sensitive measuring instruments. In adapting this form to the present work certain important modifications in the electrode vessel, in the shaking apparatus and in the bath were found advantageous. The electrode vessel was modified to overcome the difficulties produced by foaming of the juices. A cheaper and more convenient shaking apparatus was devised. A less expensive bath which is also more convenient was used. Although the electrode vessel is not maintained at a constant temperature, the slow fluctuation of the temperature of the air makes possible a temperature correction without appreciable error.

Electrode vessel

A 30-cc. dropping funnel L is attached to the hydrogen electrode vessel H as shown in the figure. The dropping funnel is attached with rubber tubing to a small glass tube which passes through the stopper J that fits into the electrode vessel. The funnel is further supported by means of a wire loop M fastened to and extending from the support N holding the electrode vessel. When the funnel is removed a pinch-cock is placed on the rubber connection to close the outlet. Plant juices, excepting those of relatively high acidity, foam considerably when hydrogen is rapidly passed through them, which results in the expulsion of considerable liquid from a small cell like the Clark vessel. By placing the liquid in the dropping funnel which is considerably wider than the cell, loss during saturation with hydrogen is prevented. An additional advantage of this dropping funnel is that it greatly lessens the period of contact between the liquid and electrode, and probably facilitates saturation of the electrode by preventing the formation of a film over its surface.
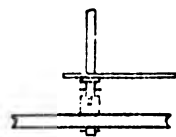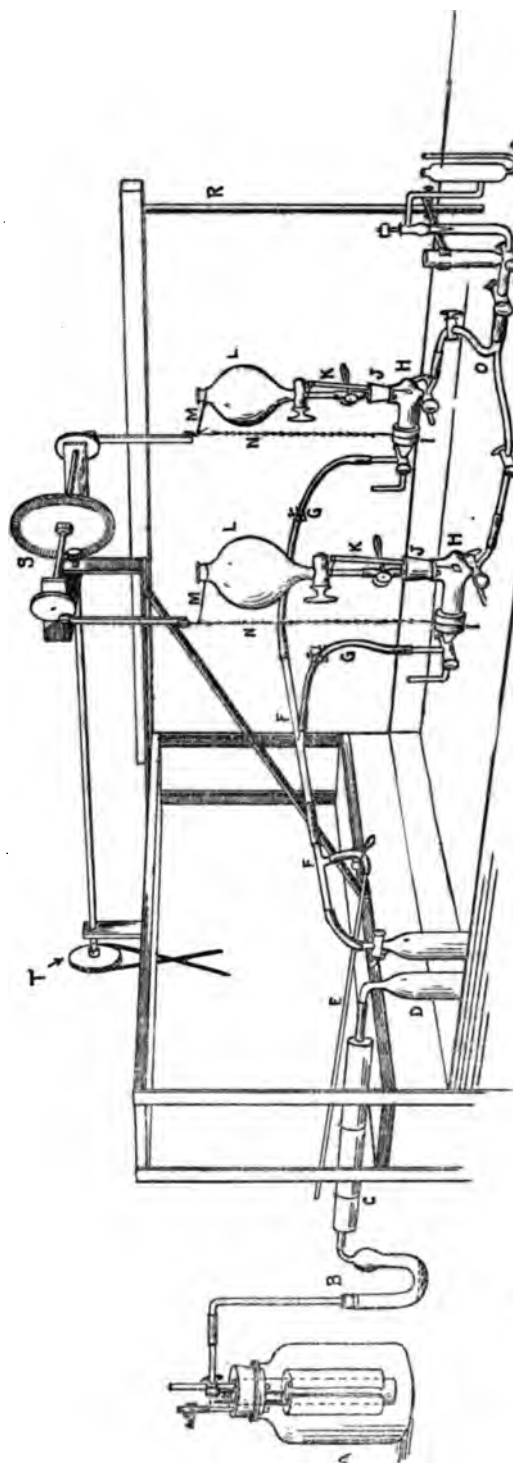
SIDE VIEW OF T

A - Hydrogen Generator
B - Tube containing H_2SO_4
C - Electrical heating unit
D - U-Tube for cooling hydrogen
E - Hydrogen overflow
F - T-Tubes
G - Hydrogen Leads

H - Clark Electrode vessels
I - Rubber Rings
J - Rubber Stoppers
K - Hydrogen Electrodes
L - Dropping Funnels
M - Funnel Supports
N - Electrode vessel supports

O - Y-Tube
P - Intermediate vessel of KCl
Q - Calomel Electrode
R - Support for P
S - Bevel Gear of Shaking Device
T - Metal Pulley

FIG. 1. THE MAIN PARTS AND THEIR ARRANGEMENT OF THE HYDROGEN ELECTRODE APPARATUS

At the place of suspension each electrode vessel is provided with a rubber ring I for insulation. Each electrode vessel is suspended by a chain N to the metal wheels mounted on the ends of a shaft 4½ inches long. This shaft is driven by a bevel gear as shown in the figure at S. In order that the shaking apparatus may be started and stopped without stopping the motor which also runs the water stirrer in the bath, the metal pulley T is arranged to slide in and out of gear as shown in the figure. The cross pin in the shaft fits into notches on the inside collar of the pulley. By pushing the pulley inward the shaking apparatus is set in gear and by pushing it outward the apparatus is put out of gear. The rate of shaking of the electrode vessel should be about one cycle per second.

Connecting vessel

The duplicate electrode vessels are joined by a glass Y-tube O to the same intermediate vessel P of saturated KCl solution. This vessel is held in position by the rigid support R. A cord supporting the Y and attached to an arm from R is not shown in the figure. The Y-tube is provided with a cock in each of the two arms.

Constant temperature bath

A constant-temperature air-bath is not used. The hydrogen electrode element and connecting vessel of the chain are suspended above a constant-temperature water-bath maintained at 25°C. In this bath the calomel electrode battery and comparison cell are kept. Since water is a fairly good conductor and stray currents from the wires, motor and other sources may play upon the bath, it is not desirable to operate the hydrogen vessel in the water. Electrical connection is often made between the chain and the water, and disturbances might easily be set up. At each measurement the temperature of the air above the bath is taken and correction made.

Calomel electrode battery

The calomel electrode battery[3] which is not shown in the figure is made up of five cells with a comparison cell Q joined by a ground-glass connection. These cells are closed with cork stoppers which are covered with a mixture of paraffin and beeswax to make tight seals. In the preparation of the chemicals the procedure given by Loomis and Acree (11) was followed. Mercury redistilled twice and N/10 KCl solution were used.

The comparison calomel electrode Q fits into the intermediate vessel by a ground-glass joint. During a measurement it is kept at constant temperature by placing it in a jar of water which sets in the bath and is raised sufficiently for this purpose.

[3] Devised by Dr. N. E. Loomis for the Eli Lilley Company.

Hydrogen generator

The hydrogen is generated electrolytically in A from a 10 per cent solution of KOH, nickel electrodes being used. Ten to twelve amperes of current are employed. This amount generates sufficient hydrogen for saturation of duplicate samples at the same time. The hydrogen first passes over H_2SO_4 in the glass tube B, then through heated palladiumized asbestos in C and finally through a glass U-tube D. The latter extends into the constant-temperature bath by which means the hydrogen is cooled. By means of two small T-tubes following the U-tube, a lead G to each hydrogen electrode vessel and an outlet E for waste hydrogen are provided. The latter outlet consists of a glass tube of smaller bore than the T and is closed by a pinch-cock at the rubber connection near the T. A glass cock in the connection from the U-tube closes the system when not in use. It was not found necessary to keep a small current running overnight to prevent diffusion of air into the generator. Although some hydrogen diffuses out from the system, any effects due to entrance of air when the sytem is closed, have not been noticed. All rubber connections are made tight by a coating of paraffin and beeswax.

Preparation of palladium electrodes

Platinum electrodes coated with palladium black are used. The black is deposited by electrolysis from a 2 per cent solution of $PdCl_2$ containing a trace of lead acetate. When sufficient black has been deposited the electrodes are placed with the current running, first into a dilute solution of H_2SO_4. This electrolyzes $PdCl_2$ adhering to the black and removes any occluded chlorine by displacement with hydrogen. They are then dipped into a dish of distilled water. The whole process of preparation requires about $1\frac{1}{2}$ minutes. Finally, the blackened electrode is thoroughly rinsed with running distilled water and is then ready for use.

In order to keep the $PdCl_2$ solution, which is used for depositing the black, free from contamination of substances from the plant, the black from the electrodes is not returned to the solution. It appears that in work with inorganic solutions special precautions (11) with regard to keeping the electrolyzing solution free from contamination are not necessary. With material like plant juices it seems that contamination from them might make it difficult to secure good electrodes. In cleaning, the electrodes are first rinsed to remove adhering plant juice and then let stand in a beaker of distilled water. This dissolves out further amounts of juice. The black is wiped off with a clean piece of ashless filter paper and the electrode is ready for recoating. At frequent intervals the electrodes are cleaned by placing in concentrated HNO_3. This dissolves the palladium, leaving a clean platinum surface.

Measuring instruments

The measuring instruments consist of a Leeds and Northrup improved type of potentiometer, a sensitive D'Arsoval galvanometer and two Leeds and Northrup Weston cells. One of the latter was compared with a cell standardized by the United States Bureau of Standards. The value of the comparison calomel electrode was checked frequently with electrodes of the battery.

PROCEDURE OF MEASUREMENT

Preparation and flooding of electrodes

Freshly prepared electrodes are always used in making a determination. These are placed into the electrode vessels and the dropping funnels with pinch-cocks are then attached. The cocks in the funnels are closed, and the vessels, tilted so the inlets are higher than the outlets, are flooded with hydrogen. The electrode vessels are flooded separately by running the hydrogen into each for about five minutes.

Extraction of plant juices

During this interval the plant material is usually cut and the sample of juice extracted. The plant tissue is first macerated by passing through a tinned meat chopper. The macerated tissue is placed on a clean muslin cloth which is spread on the bottom plate of the press which is used. The edges of the cloth are folded over each other enclosing entirely the tissue. The juice is then pressed out by means of pressure. The pressure should be uniform for different samples. The cloth should be a good grade of bleached muslin thoroughly washed and cut into squares of convenient size. A square is used but once. The cloth retains coarse colloidal and other matter, giving a juice from which on centrifuging for 10 minutes no material except salts are thrown out. The centrifuging is thus omitted.

Saturation of juice with hydrogen

Three to five cubic centimeters of the juice are placed in each of the dropping funnels. The waste outlet of the electrode vessel is closed and the cocks in the funnels opened slightly. The hydrogen is made to bubble evenly through the juice by regulation of screw clamps on the rubber leads to the vessels. The hydrogen should bubble through for about 10 minutes with the amounts of juice indicated.

No difficulty is experienced from foaming with this arrangement except when the juices approach the neutral or alkaline point. In such cases a long peaked cone of filter paper inserted snugly into the opening of the funnel is of material help. This serves to break the bubbles, permitting the hydrogen to escape. Ashless filter paper is used and pieces made by cutting the 9-cm. size in halves are convenient.

Transfer of juice to the electrode vessels

After saturation the cocks in the funnels are closed and the current pro-
ducing the hydrogen is reduced from 10 amperes to about 1 ampere. This
reduces the large hydrogen stream which is now not needed and the hydrogen
generator is not unnecessarily heated. The exit for overflow of hydrogen is
opened by releasing the pinch-cock. The cocks in the funnels are opened
and the juice allowed to flow into the electrode vessels. The cocks are closed
when a little liquid still remains in the stems of the funnels which prevents
the possibility of the entrance of air into the vessels.

Shaking electrode vessels

The electrode vessels are gently shaken for 2 minutes. Long violent
shaking sometimes appears to discharge the electrodes, while with no shaking
a local equilibrium may be obtained, as is indicated by a higher E. M. F. of
the chain. The period of 2 minutes gave good results and was arbitrarily
chosen.

Making the reading

In making the measurements only the N/10 calomel electrode is used as
shown by the following combination:

$$PdH_2 \text{ Plant Juice}//\text{Saturated KCl}//\text{N}/10 \text{ KCl HgCl Hg}$$

The electrodes are left at least half exposed to the hydrogen gas. Fresh
saturated KCl solution is run into the connecting tube of the electrode vessel
as in the usual procedure (2). A rubber tube carries away the overflow of
KCl solution from the outlet. The calomel electrode and the wires leading
to the potentiometer are put into place. Contact between the juice and the
saturated KCl is made by the film of KCl solution around the stopcock, which
is left ungreased around its middle portion. A good contact is insured if a
scratch connecting with the bore is made around the cock. Readings are
then taken immediately. Constant potentials are maintained most generally
for 2 to 5 minutes and often much longer. Duplicate measurements usually
agree within 0.1 millivolt.

Although more contact potential is ordinarily set up with small contact
surfaces (5) yet this may be the opposite when working with plant juices.
In several trials when the contact was brought below the bore of the cock
into the large tube a contact potential developed quite rapidly. This rapid
development of contact potential with freshly extracted plant juices may be
due to the reaction of the KCl with the plant juice. The N/10 KCl of the
calomel cell which comes in contact with the saturated solution is renewed
after each measurement.

The acidity of plant juices may change appreciably on standing, as is indicated in the second part of this paper; hence it is necessary to make the measurements within as short a time as possible. Since two sets of comparative determinations can be made in an hour, corrections for changes in vapor and barometric pressure (13) have not been taken into account.

Accuracy of the method

One of the tests which gives value to a method lies in the reproducibility of results. This is particularly true where comparative results are wanted. Not only should duplicate measurements, which are made at the same time and thus under the same conditions agree, but measurements made at different times also should agree. In the case of the buffer solution of KH_2PO_4 + NaOH, measurements at different temperatures, as given in table 1, show good agreement. Examples of measurements and constancy of potentials, which are respresentative of the measurements of plant juices of the following paper, are given. The good duplication of readings between the two hydrogen electrodes in separate cells with the same juice is to be especially noted.

SUMMARY

The importance of the acidity or hydrogen-ion concentration of plant juices, and the need of developing an accurate method for its determination have been pointed out.

An accurate method for this purpose has been developed in which are used: The Clark electrode vessel, modified and adapted for work with plant juices, a simple means for shaking the vessel, a constant-temperature water-bath in which the calomel electrodes are kept, electrolytically prepared hydrogen, and accurate measuring instruments. Corrections for temperature of the air are made when readings are made. Contact of the electrodes and plant juice during saturation with hydrogen is prevented by placing the latter into dropping funnels attached to the electrode vessels. By this means contamination of the electrodes by the juice during saturation with hydrogen is prevented. The electrodes are always recoated for each determination.

The plant juice is prepared by macerating the tissue and expressing the juice by means of a press. The macerated tissue is wrapped in a clean muslin cloth, which gives a juice free from coarse colloidal or other material.

Contact is made between the plant juice and saturated KCl solution by means of a scratch around the cock connecting the two. This reduces the contact potential which develops quite rapidly when the connection is made by opening the cock wide.

Duplicate measurements usually agree within 0.1 millivolt and constant potentials are maintained for several minutes or longer. A table showing the possible accuracy is given. These results are undoubtedly more accurate than any previously reported on plants.

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HYDROGEN-ION CONCENTRATION OF PLANT JUICES

II. Factors Affecting the Acidity or Hydrogen-ion Concentration of Plant Juices[1]

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INTRODUCTION

The importance of the hydrogen-ion concentration of plant juices was noted in the first part of this paper. Since many of the important reactions and processes occurring within the plant are influenced by slight changes in acidity, it is desirable to know something of the factors which produce such changes. In plant juices as in any medium the hydrogen-ion concentration depends upon the kind and amounts of acids, the nature of the solvent or medium and the buffer substances present. The hydrogen-ion concentration which an acid can produce depends upon its degree of dissociation and upon the amount of acid that is present. Organic acids are, as a rule, much less dissociated than inorganic acids and hence with equal dilutions of equivalent amounts of acids less acidity is developed by the former than by the latter.

Explanation of buffer action

Buffer substances act as regulators of the reaction preventing rapid fluctuations due to an increase or decrease in total acids. Buffer action may be illustrated in the following way. In a solution of HCl there is, according to the mass-action law, an equilibrium between the ions and the undissociated molecules which may be represented by the equation:

$$\frac{C_{H+} \times C_{Cl-}}{C_{HCl}} = K$$

This equation states that the product of the concentrations of the hydrogen and chlorine ions divided by the concentration of the undissociated HCl is

equal to a constant K. If to the solution more HCl is supplied the concentration of HCl molecules is increased and since the value of K is constant it is necessary, if the equation is to hold true, that the product of the concentrations of the hydrogen and chlorine ions also be increased. In order that the product may increase, more of the HCl dissociates to give greater concentrations of both the hydrogen and chlorine ions. If however, a salt of a weak acid as CH_3COONa is added to the solution the concentration of the hydrogen-ions is held down to some extent when more acid is added. Sodium chloride is formed and the acetate-ions which are supplied unite with hydrogen-ions present to form CH_3COOH molecules which dissociate much less than HCl molecules. Because of this slight dissociation of CH_3COOH the concentration of hydrogen-ions is lowered in an extent which depends upon the amount of CH_3COONa which is present. The CH_3COONa serves as a buffer in this case.

In plants organic salts, proteins and other substances may serve as buffers in the way just explained. Hempel (10) has investigated the buffer processes in succulent plants. She concludes that on the acid side of the litmus end-point, normal malate is the main buffer and on the alkaline side of the litmus end-point aluminum malate and some other unknown substances are the principal buffers. These buffer substances play a very important rôle in plant life because they prevent rapid and excessive changes in the acidity of plants which would otherwise result from factors mentioned in the following discussion.

Acids found in plants

According to Pfeffer (20) every plant produces some organic acid either free or in the form of an acid salt. Oxalic, malic, citric, and tartaric acids occur most frequently. With the exception of the oxalic acid present as calcium oxalate, the organic acids and their salts are for the most part held in solution. The inorganic acids present in the plant are taken up from the soil. The distribution of the acids in the plant (1) has been found to vary with the different parts as stems, leaves, and flowers.

Formation of organic acids in plants

The formation of organic acids in plants is now commonly held to be connected with the cell respiration processes. Pfeffer (20) states that the acids are usually products of katabolism, but probably may also be produced synthetically. Kraus (13) believes that these acids are secondary products from the splitting up of proteins. Astruc (2) states that production of acids is intimately associated with processes of respiration and assimilation. The acidity in succulents he says is due chiefly to malic acid. Later workers, as Long (15), MacDougal, Long and Brown (17), hold the view that acids are formed from the metabolism of carbohydrates. Factors, as water supply,

light, and temperature, which influence the formation of carbohydrates and sugars will indirectly influence the production of these acids. In succulent plants MacDougal, Richards and Spoehr (18) attribute acid formation to imperfect oxidations resulting from the lessened aeration in the interior of massive tissues. Considerable work has been done by Richards (22, 23, 24, 25, 26) on acidity and the gas interchange of oxygen and carbon dioxide in cacti. Richards (25) states that the partial oxidation processes which give rise to acids of which undoubtedly the chief one is malic can be conceived as taking place at all times. The substance oxidized is believed to be the complex of the living protoplasm itself, which in turn draws upon the reserve food supply of accumulated carbohydrates. It is likely that sugars form unstable compounds with the living protoplasm and in such unions oxidation and release of energy take place. Whether free oxygen is necessary for this process Richards does not know, but quotes Astruc (25) as stating that acids are best produced in an atmosphere rich in oxygen. Purjewicz (21) has pointed out that in the breaking down of acids which he regarded as an oxidation process, in the absence of oxygen this process is greatly inhibited. It appears thus that free oxygen is needed for both processes. However, Richards (25) is inclined to doubt that free oxygen is needed for succulents in which cases auto-oxidations by intramolecular respiration might occur.

An early view of acid formation, but which found little favor among investigators, was that of Liebig (14). Liebig thought from the disappearance of organic acids in the ripening of fruits, that these acids were concerned in the building up of carbohydrates and constituted intermediate products between the carbon dioxide on the one hand and sugar on the other. In view of the fact that acids are formed and accumulate within the plant during the night when photosynthesis of carbohydrates does not occur, this theory at once becomes untenable.

Concerning the period of growth of the plant in which most acidity is produced, Aubert (3) found less acid in the young parts and most in the recently mature tissue. According to Astruc (2) the acids are formed most in young organs which show high cellular activity and a maximum of turgescence. The acids gradually diminish in amount as the tissues advance in age either by uniting with bases absorbed from the soil or by esterification. Nicolas (19) states that "in young organs, principally leaves, intramolecular combustions are more complete than in older organs; young tissues consume much more oxygen than those completely developed, ... and thus liberate greater quantities of energy which they use in growth." This statement is in harmony with the observations of Aubert just noted as to formation of acids if they are produced from incomplete oxidation processes.

Acid formation in plants appears to be intimately connected with the living protoplasm. Although the acids may be largely derived from transformations of carbohydrates, it seems that for breaking up of carbohydrate molecules with liberation of energy the molecules must first form some sort of union

with the living protoplasm. How far the processes for securing energy from the carbohydrates are removed from those in which the proteins are formed and broken down is not known. In all these processes the protoplasm is concerned. Whether acid formation is regarded as a result of incomplete oxidation of carbohydrates or from the formation and decomposition of proteins or all of them depends largely upon the way in which the metabolic processes of the plant are viewed.

Factors influencing acid accumulation

The factors which influence the accumulation of acids may be divided into two groups: viz., internal and external factors. Considering the internal factors, the accumulation of acids depends upon the kind and age of the plant and the removal of the acids by oxidation or precipitation as salts. The chemical reactions of the plant are governed by the same factors affecting chemical reactions in the laboratory, such as temperature, concentration, and nature of the reacting substances. The formation of calcium oxalate is an example of the removal of an acid as an insoluble salt. Salts of other acids are usually more soluble and their production occurs to some extent in a self-regulatory manner, since in the higher plants they are commonly used in the maintenance and regulation of turgor (20). Pfeffer (20) states that a diminution in acidity of the cell sap may be produced by an increased formation of alkali, by a consumption of acid, or by union with basic substances.

The external factors which influence most the accumulation of organic acids are light and temperature. That high illumination and high temperature produce rapid destruction or decomposition of these acids has been found by many investigators. Heyne (11) was the first to note that leaves had a more acid taste in the morning than late in the afternoon. Kraus (13) attributed the loss of acid during the day to the direct effect of light. De Vries, who worked upon the periodicity of acid formation in succulents, is reported (25) to have found that prolonged darkness as well as exposure to high temperatures produces a loss of acids. De Vries regards the formation and splitting up of the acids as coincident processes which are always taking place, the former being more rapid than the latter at night. Warburg (31) believed that the decomposition of the acids during the day furnished a source of carbon dioxide for photosynthesis in plants which were not favorably situated as to gas-interchange relations. Aubert (3), Purjewicz (21) and Astruc (2) also found this periodicity of formation and destruction of acids to be due to light and temperature changes. Pfeffer (20) states that the percentage of acids decreases to about the same extent when the temperature of a plant in darkness is raised from 15° to 45°C. as when it is exposed to daylight.

Probably the work of Spoehr (27, 28, 29) on the photolysis of plant acids ~~has~~ been the most extensive as regards their chemical decomposition. Spoehr ~~in~~ working in vitro with chemical preparations found that acetic, glycolic, ~~malonic,~~ malic, tartaric and citric acids are decomposed in light, giving off

considerable quantities of carbon dioxide. In general the acids in the form of salts are broken up more easily. The decomposition of malic acid and its products was studied (27) in particular. This acid gave the following products: formaldehyde, acetaldehyde, formic acid, acetic acid, glycolic acid, oxalic acid, and carbon dioxide. It is thought that in the plant these products can also be formed by the influence of light. From the malic acid, carbon dioxide is split off, giving rise to alcohols which in presence of air are oxidized to aldehydes and these, in turn, to acids. In this way the formation of oxalic acid may be explained. Although the latter acid is easily decomposed by light, its calcium salt is photochemically a stable compound. It is also pointed out that increases in volatile acids, as acetic and formic, can be explained in this way.

With plant juices Spoehr found that deacidification is not due to action of enzymes, neither is it wholly dependent upon the living protoplasm, since expressed juice when placed in sunlight diminishes in acidity with formation of carbon dioxide. Whether the latter conclusion is correct may be questioned since fresh plant juice undoubtedly contains living protoplasm.

Long (15), in working with large cacti, found a higher accumulation of acids in the outer than in the inner regions. This higher amount of acids, he says, is probably due to the higher concentration of sugar in the outer parts. In these parts sugar metabolism is greatest and hence a greater production of acids results. Since the outer parts are most exposed to the heat and light, it is here also that the greatest fluctuations due to diurnal changes occur. In what manner the acids are produced in the metabolism of sugars is not made clear.

The effect of the kind of soil and its fertilizer treatment on plant acidity is beginning to receive considerable attention. The effect and relation of the soil medium upon the plant (12) and acidity within the plant appears to be of considerable importance. Reference to this relation has already been made in Part I of this paper (page 217) in referring to the investigations of Truog and Meacham. Charabot and Hebert (5) found that with the peppermint plant, mineral salts when added to the soil generally increased the amount of volatile acid in the fresh leaves.

The effect of soil treatment is further shown in the work of Bauer and Haas (4). Their results indicate that there is a direct correlation between the acidity of the soil and that of the corn plant.

The observation of Comes (7) of the relation existing between the acidity of the cell sap of wheat and its resistance to rust is of interest. Comes found that with an increase in starch in the grain there is less acidity in the cell sap, making the plant more susceptible to rust attacks. Since with the increase in amounts of starch the percentage content of protein is lowered, this observation strengthens the view that acids are formed as by-products in protein metabolism. It is thus evident that factors like soil treatment, which influence the production of starch, may have an effect upon the acidity.

EXPERIMENTAL

The measurements reported herein were all made by the method and apparatus described in Part I of this paper. The factor studied first was the effect of time of standing of expressed juice and of plants which had been cut. In work of this nature it is important to know something of the rapidity of the changes in acidity due to cutting of the plants and standing of the extracted juice in order to eliminate errors from these sources.

Effect of standing on acidity of juice and plants

The results of the effect of standing on the acidity of extracted juice are given in table 1. Alfalfa juice was used. This table shows that the extracted juice suffers a change on standing which may be in either direction. In table 1

TABLE 1

Effect of standing on the acidity of plant juice expressed in P_H values

(a) Plants cut 2:35 p.m.

Time of standing, minutes	0	80
P_H value	5.935	5.930

(b) Plants cut 2:00 p.m.

Time of standing, minutes	0	45	135
P_H value	5.790	5.816	5.758

(c) Plants cut at 7:15 a.m.

Time of standing, minutes	0	50	115
P_H value	5.900	5.886	5.753

(a) a slight increase in acidity has occurred. In table 1 (b) the juice is shown to become less acid during the first hour of standing. This is followed by an increase in acidity 90 minutes later, which acidity is greater than that of the fresh juice. In table 1 (c) the acidity increases directly on standing.

This increase in acidity is probably due to enzyme action on the sugars and other changes in the protoplasmic substances present. The decrease in acidity of the juice in table 1 (b) is probably due to the breaking up of acids present as noted by Richards (25). In this case it appears that at first the rate of acid destruction was greater than the rate of acid formation.

The changes in acidity of the juice of plants which have been cut and let stand in the laboratory were uniformly toward less acidity, with one exception. Table 2 gives these results. It is to be noted that these changes correspond to the diurnal changes in acidity of plants during the day due to such factors as light and temperature.

The results in tables 1 and 2 show that acidity changes do occur in extracted juices and plants that have been cut.   Although these changes may be small, yet it is clearly shown that determinations of acidity should be made as quickly as possible after the plants are cut and the juice is extracted.

TABLE 2

The effect on acidity of allowing cut alfalfa plants to stand before expressing the juice

(a) Plants cut 2:15 p.m.

Time of standing, minutes	0	70	145
P_H value	5.920	5.973	6.050

(b) Plants cut 8:20 a.m.

Time of standing, minutes	0	95
P_H value	5.802	5.907

(c) Plants cut 8:30 a.m.

Time of standing, minutes	0	90
P_H value	5.711	5.788

(d)

Time of standing, minutes	0	100
P_H value	5.935	5.987

(e)

Time of standing, minutes	0	105
P_H value	5.790	5.956

(f)

Time of standing, minutes	0	170
P_H value	5.900	5.793

Effect of diurnal changes on the acidity of the juice from cowpeas

The acidity changes in the juice from the leaves, stems, and roots of cowpeas were followed for a period of 24 hours.   The cowpeas were grown in the greenhouse in a large cylinder containing a slightly acid sand soil and were 32 days old when the determinations were made.   Inoculation was insured by adding to the soil a culture of the proper legume bacteria.   Determinations of acidity of the leaves and stems were made alternately every two hours, starting at 6.30 a.m.   The acidity of the roots was determined but four times during the 24-hour period.   The results of these determinations are given in table 3.

TABLE 3

*Diurnal changes in acidity expressed in P_H of juice from leaves, stems, and roots of cowpeas.
Determinations started 6:30 a.m., July 25, 1919*

TIME OF DETERMINATION	LEAVES	STEMS	ROOTS
	P_H	P_H	P_H
6:46 a.m	5.400		
7:38 a.m.		5.047	
8:32 a.m	5.417		
9:24 a.m.		5.076	
10:04 a.m.			5.834
10:37 a.m.	5.276		
11:26 a.m.		5.166	
12:42 p.m.	5.369		
1:22 p.m.		5.186	
2:47 p.m.	5.426		
3:43 p.m.		5.210	
4:41 p.m.			5.742
4:57 p.m.	5.517		
5:45 p.m.		5.319	
6:40 p.m.	5.499		
7:42 p.m.		5.294	
8:25 p.m.	5.529		
9:41 p.m.		5.323	
9:05 p.m.			5.741
10:43 p.m.	5.607		
11:23 p.m.		5.314	
12:32 a.m.	5.813		
1:37 a.m.		5.216	
2:27 a.m.	5.451		
3:56 a.m.		5.186	
3:13 a.m.			5.769
4:31 a.m.	5.451		
5:12 a.m.		5.151	

In figure 1 these results are plotted as curve with the time of day as abscissas and the P_H values as ordinates. These curves show a periodicity in P_H for the different parts of the plant.

It is to be noted that in the leaves the highest acidity occurs in the morning, from which time on the acidity decreases. This is in accord with the results of previous investigators to which reference has been made. In case of the stems, the curve shows the same general trend as in case of the leaves.
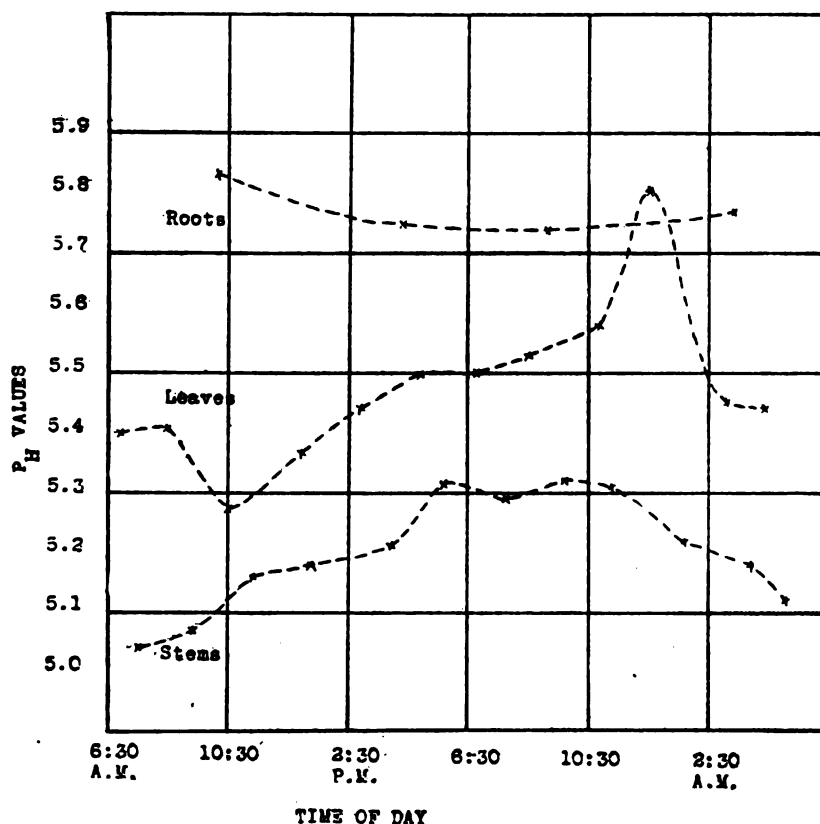
FIG. 1. CURVES SHOWING DIURNAL CHANGES IN ACIDITY OF LEAVES, STEMS, AND ROOTS OF COWPEAS

Acidity determinations started 6: 30 a.m., July 25, 1919

The acidity gradually decreases during the day, reaching its lowest point about 9.30 p.m. from which time on it gradually increases. The curve for the roots indicates a reverse in acidity changes from those of the leaves and stems. The time of highest acidity in the roots appears to occur in general during the day. This may possibly be due to the faster removal of the bases from the roots during the day-time because of a greater transpiration stream.

Effect of soil on acidity of plant juice

Crops of oats, buckwheat, soybeans, and cowpeas were grown on a strongly acid (30) sand soil receiving different treatments and the P_H values of the juices of the plants were determined at different stages of their growth.

The plants were grown in the greenhouse in pots containing 11,500 gm. of Plainfield sand. Each pot received 2 gm. of K_2HPO_4 added in solution and mixed thoroughly through the soil. One-half of the pots received in each 36.8 gm. of finely ground limestone, which was at the rate of about 4 tons per acre, and was mixed into the soil at the same time with the K_2HPO_4.

For growing the plants which were used in their early stages of growth, pots containing one-half the amounts of soil stated were used. Fifty-six pots in all were used for the four kinds of crops. The jars were seeded on June 19. In order to determine what the natural or normal acidities of seedlings of these plants might be when unaffected by soil nutrients, seeds of these plants were at this same time germinated in pure white quartz sand without the addition of nutrients.

TABLE 4

Acidity of stems and leaves of seedlings grown in quartz sand without nutrients. Seeded June 19, 1919

DATE OF DETERMINATION	P_H VALUES OF PLANTS			
	Oats	Buckwheat	Soybeans	Cowpeas
June 27............				5.724
July 1............	5.576	4.332	5.965	
July 4............	5.534			5.648
July 8............			6.119	

On June 23 the soybeans and cowpeas were inoculated with the proper legume bacteria and to each of the large jars of oats and buckwheat 0.5 gm. NH_4NO_3 was added in solution. The small jars received 0.25 gm. each. On July 13 a second application of K_2HPO_4 of 0.5 gm. in solution was added to each of the large jars.

Differences in the size of the plants due to the favorable action of the lime were noticeable in case of oats and buckwheat on June 26, which difference had disappeared by July 13. Soybeans responded to lime and a distinct difference between the limed and unlimed jars was maintained throughout the growing period. The cowpeas showed no increase in growth due to lime, but the plants of the limed soil were darker green in color. In fact lime produced a darker green color in all the plants. At the time of the last determinations of the acidity of the plants, the oats and buckwheat were in the flowering stage but the soybeans and cowpeas had not yet reached that stage.

In table 4 the acidities of the juice of the seedlings grown in white quartz sand are given. In case of the soybeans and cowpeas the cotyledons were discarded.

In tables 5, 6, 7, and 8 are given, respectively, the acidities at different stages of growth of oats, buckwheat, soybeans, and cowpeas grown on un-limed and limed Plainfield sand. Acidities in different parts of the plants also are given.

TABLE 5

Acidity of the oat plant at different stages of growth and in its different parts. Oats seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
July 1	Whole top	5.812	5.783
July 4	Whole top	6.009	5.859
July 10	Whole top	5.938	5.896
July 14	Whole top	6.102	5.955
July 22	Whole top	5.843	5.518
July 28	Leaves	5.968	5.722
July 28	Stems	5.954	5.854
July 28	Roots	6.197	6.368

The results in table 5 on oats show that the leaves and stems of the limed plants were more acid than those of the unlimed plants. The roots, however, were more acid in the case of the unlimed plants.

TABLE 6

Acidity of the buckwheat plant at different stages of growth and in its different parts. Buck-wheat seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
July 1	Whole top	4.832	5.429
July 8	Whole top	4.696	4.878
July 10	Whole top	5.003	4.823
July 13	Whole top	5.170	5.219
July 18	Whole top	4.949	5.205
July 20	Leaves	5.362	5.931
July 20	Stems	5.153	4.968
July 28	Leaves	5.284	5.755
July 28	Stems	4.237	4.378

In table 6 the acidity of buckwheat plants is shown in general to decrease where lime was added. Only in two cases did the limed plants show a greater acidity. The acidity of the roots was not determined because of insufficient material.

In table 7 the results for soybeans show that where the whole tops or leaves are compared, the limed plants had the greater acidity. The stems of the limed plants were less acid in two of three cases. The roots of the limed plants as in the case of oats were also less acid.

TABLE 7

Acidity of the soybean plant at different stages of growth and in its different parts. Soyt seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limec
July 1	Whole top	5.970	5.94
July 8	Whole top	6.246	6.14
July 13	Whole top	6.408	6.25
July 14	Stems	6.092	6.17
July 22	Leaves	6.533	6.27
July 22	Stems	5.981	5.79
July 28	Leaves	6.272	6.24
July 28	Stems	5.827	5.84
July 28	Roots	5.947	5.99

Table 8 gives the results with cowpeas. The leaves of the limed pl are the more acid as is the case of the leaves of the limed soybeans and c With the tops and leaves together the limed plants are less acid, with exception. It is to be noted again that the roots of the limed plants are acid, as were those of oats and soybeans.

TABLE 8

Acidity of the cowpea plant at different stages of growth and in its different parts. Cow seeded June 19, 1919, on Plainfield sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES	
		Unlimed	Limed
June 27	Whole top	5.685	5.76
June 30	Whole top	5.518	5.53
July 8	Whole top	5.471	5.45
July 12	Whole top	5.659	5.73
July 22	Leaves	5.811	5.63
July 22	Stems	5.431	5.34
July 27	Leaves	5.703	5.55
July 27	Stems	5.552	5.65
July 27	Roots	5.785	5.83

In a study and comparison of the data from the four crops, several ul pected results occur. There is, however, a direct correlation between and plant-root acidities. The acidity of the buckwheat roots could no determined because the amount of material was too limited. Howevel seems safe to conclude that the roots of buckwheat plants grown on lil soil were less acid, as was the case with the stems and in most cases with leaves. This correlation between plant-root acidity and soil acidity is important one, and is what normally would be expected. It indicates t an acid condition of the soil may limit the supply of bases to an extent suffic

to raise the plant-root acidity. The rise in plant-root acidity undoubtedly limits the supply of bases needed by the growing stems and leaves. The rise in root acidity may also act unfavorably on the symbiotic bacteria which live in nodules attached to the roots of the legumes. This would be in direct correlation with the results of Fred and Loomis (9) and Fred and Davenport (8) which show that legume bacteria have a rather narrow optimum range of acidity.

In the case of the leaves of the different plants, with the exception of buckwheat, the results are the reverse of what they are with the roots. From these results the effect of lime on the acidity of leaves may be explained as follows. Since the effect of lime is in general to produce healthier and more vigorous plants, it is possible that in some cases these plants may have a higher acidity than those not receiving lime. In healthier and more vigorous plants metabolic processes are taking place more rapidly and if the production of acids is a measure of protein or carbohydrate metabolism, the larger amounts of acids in the leaves can be explained, even though the supply of bases in the roots of limed plants is greater. However, to establish this point more

TABLE 9

A comparison of effects of NH_4NO_3 and $NaNO_3$ on the acidity of oats grown on unlimed and limed acid Plainfield sand. Oats seeded July 3, 1919

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P_H VALUES			
		NH_4NO_3		$NaNO_3$	
		Unlimed	Limed	Unlimed	Limed
July 16.....................	Whole top	5.877	5.857	5.829	5.777
July 19.....................	Whole top	5.910	5.926	5.950	5.881

fully it would be necessary to correlate by plant analysis the protein and carbohydrate content, and perhaps ash content, with the plant acidity. Much information could probably also be obtained from comparisons of the diurnal changes in acidity of limed and unlimed plants.

To the plant cultures with Plainfield sand, NH_4NO_3 was added in order to insure an ample supply of nitrogen. Undoubtedly in the unlimed soil the ammonium part of the salt did not change to nitrates as rapidly as in the limed soil and hence there was the possibility that this ammonium might lessen the acidity of the plants. In order to get some information regarding this, oats were grown on Plainfield sand supplied with the same treatment as previously given, with the exception that in one set the nitrogen was supplied as NH_4NO_3 and in the other as $NaNO_3$. The results are given in table 9. The acidity of the plants was less in the case of the unlimed soil when either sodium or ammonium nitrate was used, with one exception. With the exception of one case the plants receiving ammonium nitrate were less acid than those receiving sodium nitrate. These results indicate that fertilizers may have an appreciable effect on plant acidity.

In table 10 a comparison of the effects of sodium and ammonium nitrates on the acidity of oat seedlings grown on quartz sand without nutrients is given. In this case the plants grown with NaNO₃ were less acid.

TABLE 10

A comparison of the effects of NH₄NO₃ and NaNO₃ on acidity of 7-day-old oat seedlings grown on quartz sand

DATE OF DETERMINATION	CHARACTER OF MATERIAL	P$_H$ VALUES	
		NH₄NO₃	NaNO₃
July 21................................	Whole top	5.658	5.750

SUMMARY

Views concerning the formation of acids within the plant and factors affecting their accumulation have been briefly reviewed. These views conflict in many cases. One of the most plausible views concerning acid formation is held by Richards (25). He supposes that the living protoplasm forms with the carbohydrates unstable unions which by intramolecular respiration processes break down, giving organic acids as by-products. Under good conditions of oxidation these would be largely broken down into water and carbon dioxide. Incomplete oxidation of carbohydrates existing in one form or another thus seems to be one of the main sources of acids other than carbon dioxide. The relation of protein metabolism to acid formation is not definitely known but undoubtedly some acids at least may be formed in these processes. Acids accumulate at night and are destroyed in the daytime, hence it seems reasonable to believe that high illumination and high temperatures bring about rapid destruction of plant acids.

In making determinations of acidity or hydrogen-ion concentration of plant juices it is very important to take into consideration the following. Expressed plant juice usually becomes more acid on standing. Plants when cut and allowed to stand some time before the juice is expressed usually become more alkaline. Where a comparison is to be made between two sets of plants, they should all be cut at the same time and the determinations made as quickly as possible.

The diurnal changes in the acidities of the leaves, stems, and roots of the cowpea were followed during a 24-hour period. In conformity with the work of other investigators using different methods, the acidity of the leaves and stems was found to be highest in the morning and decreased during the day. The acidity of the roots was found to be highest during the day. The higher acidity of the roots in the day may possibly be due to the faster rate of movement of bases from the roots up into the plant during the day because of a greater transpiration stream at this time. The decreased acidity of the leaves and stems in the day is attributed to the destruction of the accumulated acids by influences as increased light and higher temperatures. Undoubtedly diurnal changes in plant processes also are factors.

Acidity measurements were made of oats, buckwheat, soybeans, and cow-peas at different stages of their growth and in their different parts. Plainfield acid sand, part of which received sufficient lime to neutralize the acid present, was used. Many unexpected results were obtained. With the exception of buckwheat the tops of the limed plants were usually more acid. There is thus an indication that in some cases lime, by stimulating the natural life and growth processes of the plant, causes a rise in the production of acids and hence a higher acidity within the leaves where the most active processes take place.

The acidity of the roots of the plants appears to correlate with the acidity of the soil. The relation of soil acidity and liming to plant growth and the symbiotic legume bacteria is thus indicated, since the roots are the source of the lime needed by the other parts of the plant for neutralizing acids and other purposes.

The acidity of the tops of oats grown on unlimed soil is less than in limed soil when either $NaNO_3$ of NH_4NO_3 is used as a source of nitrogen, with one exception. With pure quartz the use of $NaNO_3$ produced plants of less acidity than the use of NH_4NO_3. With Plainfield sand soil the reverse was usually the case. The form of fertilizer thus appears to affect the acidity of plants to some extent.

The investigation reported has been largely on methods and hence is of a preliminary nature. Further investigations are needed before definite conclusions can be drawn regarding the influence of soil acidity and fertilizers on the acidity and metabolic processes of plants. The influences of the soil upon the acidity of the plants could undoubtedly be made clearer, if the diurnal changes of the acidities of the plants were followed in all cases. Plant analysis for protein, carbohydrate, and ash content might also show something of the relation between acid formation and the formation or destruction of these classes of compounds, as well as their relation to the ash and especially lime content.

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THE INFLUENCE OF LIME ON THE NITRIFICATION OF BARN-YARD MANURE-NITROGEN IN ARABLE SOIL

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INTRODUCTION

Experiments made by Barthel and others have shown that the part of the nitrogen in solid manure that is first nitrified in the soil is the ammonia-nitrogen. This is only what is to be expected. However, the experiments showed further, not merely that the higher-nitrogen compounds, apparently, are not nitrified until after the lapse of a very long time, but that even the ammonia-nitrogen present from the outset is not in its entirety converted into saltpetre during the first year of growth. Moreover, it was found that in well-tilled arable soil with a decidedly acid reaction, nitrification can take place with as much energy, at least, as in a soil of neutral reaction. It is true that these experiments were not made under conditions exactly similar to those prevailing in agricultural practice; but the circumstances were extremely favorable for the rapid and energetic operation of the nitrification process.

Thus previous experiments had shown that even in conditions extremely favorable to nitrification only a part, larger or smaller, of the ammonia nitrogen of solid manure is nitrified during the first year of growth; but it still remained to be ascertained whether the fertilizing action of the stable manure could be enhanced by any of the artificial fertilizers which are within the reach of the farmer.

The first factor considered was lime. From many points of view lime is a very valuable fertilizer, mainly because of its basic properties and its consequent capacity for regulating the hydrogen-ion concentration in the soil in a manner favorable to bacterial life. However, few experiments had hitherto been made with the direct object of ascertaining the action of lime on the nitrification of stable manure, and as these were by no means decisive, it was considered well worth while to take up this question for investigation.

The method principally employed was that adopted in our previous experiments on nitrification. Briefly, it was as follows. The different ingredients were first carefully blended with the soil which was then placed in glass jars holding 5 kgm. each. The soil was well packed down by knocking each jar on the floor a certain number of times, and each jar was sealed with a cork, in the middle of which was a short glass tube containing cotton-wool. By this

simple procedure the evaporation is practically reduced to nil, even if the soil is kept for a very long time, while the air has practically unimpeded access to the soil. During the experiments the jars were kept in the laboratory at a temperature of 15 to 25°C. according to the season of the year. At regular intervals samples were analyzed for their content of nitrate-nitrogen, ammonia-nitrogen and moisture.

The nitrate-nitrogen was determined colorimetrically in accordance with the phenol-sulfuric acid method. The content of moisture in the soil in our experiments remained constantly between 15 and 20 per cent, the most favorable percentage for the formation of nitrate.

A number of experiments were made in the determination of the ammonia content of the soil, and are recorded below.

DETERMINATION OF THE PERCENTAGE OF AMMONIA IN THE SOIL

As McBeth (5) has shown, ammonia which is added to the soil either in a free state, or in the form of ammonium salts, cannot be quantitatively recovered by any of the usual methods for the determination of ammonia in soil. McBeth tested the methods of both Boussingault (direct distillation with MgO) and Schlösing (extraction with HCl and the distillation of the extract with KOH) and found them to be approximately of equal value. When 50 mgm. of ammonia-nitrogen were added to 100 gm. of earth, the nitrogen losses in the case of two soils examined by McBeth amounted to 48 and 80 per cent. By direct distillation with KOH or by boiling with HCl for 4 hours and distilling the extract with KOH, the losses could be reduced considerably. However, neither of these methods seems to be desirable, as in both cases the splitting of ammonia out of the organic nitrogenous elements of the soil is to be feared. McBeth found also that the capacity of the soil for binding ammonia, with the exception of humus soils, increases directly with the depth below the surface.

The results of some experiments on three different soils are set forth in table 1. The stated admixtures of ammonia were supplied in the form of a solution of sulfate of ammonia (25 cc.), which was allowed to soak in the soil for at least 30 minutes before the determinations of ammonia were made (in accordance with the Boussingault method). The original content of ammonia in the soil was, of course, determined at the same time. The distillate amounted to 350 to 400 cc. All the analysis figures in this and the following tables are the average of two determinations. Soil III was a humus soil.

These experiments thus confirm McBeth's results, even though the losses here are much smaller. Even in this latter respect our results tally with those of McBeth in principle seeing that in the case of humus soils the losses did not increase with the depth beneath the surface.

There is thus reason to doubt the correctness of the ammonia figures obtained for original soils with the use of the ordinary method of distillation with MgO. A method for the determination of ammonia cannot in fact be regarded

as reliable unless the results of the analysis show the original ammonia content in the soil plus the ammonia added.

Baragiola and Schuppli (1) maintain that they have solved this problem. After adding 100 cc. of water to 50–100 gm. of soil, they distilled off to dryness with MgO in a vacuum of 15 mm. Hg. at 35°C. The cupellation was facilitated by an ammonia-free current of air, which was supplied through a capillary tube. In all the cases investigated they recovered quantitatively the ammonia added.

TABLE 1

Determinations of ammonia in soil according to Boussingault's method

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN ADDED TO 50 GM. SOIL	AMMONIA-NITROGEN RETAINED IN PERCENTAGE OF THE AMOUNT ADDED
	cm.	mgm.	per cent
Soil I	5–15	53.8	4.39
	25–35	53.8	7.71
Soil II	5–15	53.8	7.94
	25–35	53.8	16.28
Soil III	5–15	53.8	5.19
	25–35	53.8	3.73

TABLE 2

Determination of ammonia in soils: comparison of methods of Boussingault and of Baragiola and Schuppli

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITROGEN ADDED TO 50 GM. SOIL	LOSS OF AMMONIA-NITROGEN IN PERCENTAGE OF THE AMOUNT ADDED	
			Baragiola and Schuppli method	Boussingault method
	cm.	mgm.	per cent	per cent
Soil I	5–15	53.8	5.21	1.56
Soil II	5–15	53.8	13.42	7.94
	25–35	53.8	27.60	16.28
Soil III	5–15	53.8	2.93	15.10

Table 2 gives the results obtained in comparative experiments with the last-mentioned method and the Boussingault method, when the soil was supplied with $(NH_4)_2SO_4$.

Thus only in one case was the vacuum method superior to the ordinary distillation. In the three other cases, on the other hand, it yielded far *inferior* values to the Boussingault method. The explanation why Baragiola and Schuppli obtained such good results with their method must be that they worked with soils which were very weak binders of ammonia. It is manifest

from both tables 1 and 2 and from the figures given by McBeth that this absorbing capacity may in fact vary considerably for different soils.

The determinations of ammonia in the soils without admixture of ammonia gave the values shown in table 3.

Baragiola and Schuppli found that the soils investigated by them were entirely free from ammonia, with the exception of a strongly manured garden soil and a dry peat soil, which contained, respectively, 1.45 and 0.15 mgm. ammonia-nitrogen per 50 gm. of soil. As is evident from table 3, the vacuum method in this case too gave low values, which are considerably surpassed by the results obtained with the Boussingault method. However, as neither of the methods quantitatively showed the ammonia added, it is not possible to decide which of them gives the more correct value for the ammonia content of the soil. It is equally possible that the Boussingault method is more capable of separating adsorbed ammonia, and that the capacity of the vacuum method in this respect is inadequate, as the increased output in accordance with the Boussingault method is due to a splitting of organic nitrogen compounds.

TABLE 3

| NUMBER OF SOIL | DEPTH OF SAMPLING | AMMONIA-NITROGEN IN 50 GM. SOIL | |
		Baragiola and Schuppli method	Boussingault method
	cm.	mgm.	mgm.
Soil I	5–15	0.21	2.24
Soil II	5–15	0.07	3.50
	25–35	1.89	9.66
Soil III	5–15	0.07	8.40

There is certainly no reason to prefer the vacuum method to the Boussingault method, especially as the latter seems to release more adsorbed ammonia (table 2).

We have also tested the following method, elaborated by Potter and Snyder (8) for the determination of ammonia nitrogen in soil, a method for which entire reliability is claimed. A retort is charged with 25 gm. of soil, 50 cc. of ammonia-free water, a few drops of some heavy mineral oil (in order to prevent foaming) and about 2 gm. Na_2CO_3. The retort is in connection with a flask containing $N/10$ H_2SO_4 and an air pump. Ammonia-free air is sucked through the apparatus at a speed of 250 liters an hour.

The experiments were made on soil II from 5–15, 15–25 and 25–35 cm. depths. At the same time determinations were made according to the Boussingault method. The results are set forth in table 4.

Twenty-five grams of each of the soils contained 0.07, 0.0 and 0.0 mgm. of ammonia nitrogen, respectively, in accordance with the Potter and Snyder method, and 2.82, 3.29, and 2.73 mgm. according to the Boussingault method.

As is evident from the table, the Potter and Snyder method also, in spite of the fact that the prescribed airing period was increased to 20 hours, did not come up to promise, but on the contrary gave decidedly inferior results to the Boussingault method.

Some attempts were made also to determine the content of ammonia in soil in accordance with the Steinkopf (9) method—distillation with water vapour in a vacuum (with MgO). In this case a more effective liberation of the adsorbed ammonia was possibly to be expected without risking splitting-off of organic nitrogen compounds than with the Baragiola and Schuppli vacuum

TABLE 4

Determination of ammonia in soils; comparison of methods of Potter and Snyder and of Boussingault

NUMBER OF SOIL	DEPTH OF SAMPLING	AMMONIA-NITRO-GEN ADDED TO 25 GM. SOIL	AMMONIA-NITROGEN RETAINED IN PERCENTAGE OF THE AMOUNT ADDED	
			Potter and Snyder method	Boussingault method
	cm.	*mgm.*	*per cent*	*per cent*
	5–15	21.56	19.48	4.22
Soil II......................	15–25	21.56	17.21	12.01
	25–35	27.02	24.48	20.23

TABLE 5

Determination of ammonia nitrogen in soil II: Comparison of methods of Steinkopf, of Baragiola and Schuppli and of Boussingault

METHOD	AMMONIA-NITROGEN FOUND IN		LOSS IN PERCENTAGE OF NITROGEN ADDED
	50 gm. soil	50 gm. soil + 53.76 mgm. ammonia-nitrogen	
	mgm.	*mgm.*	*per cent*
Steinkopf........................	0.14	50.82	5.73*
Baragiola and Schuppli......................	0.07	44.59	17.18
Boussingault...............................	2.24	51.66	8.07

* After the second distillate.   After the first the loss was 11.72 per cent.

method.   An experiment with the Steinkopf method on soil II, which had proved to possess the greatest capacity for binding ammonia, gave the values shown in table 5.   In these experiments the pressure was 40 mm. Hg and the temperature of the water bath 40°C.   Two distillates were taken, each of 450 cc.   For the purpose of comparison, the corresponding figures for parallel determinations in accordance with the Baragiola and Schuppli and the Boussingault methods are included.

With regard to the separation of ammonia added, the Baragiola and Schuppli method was evidently far inferior to that of Steinkopf.   The latter vacuum method has proved to be about as effective as the Boussingault method.

It is considered (3, 6, 7) that the capacity of the soil for adsorbing ammonia, partly at any rate, is to be ascribed to zeolites. The latter contain Na, Ca or Mg, which on treatment with a diluted solution of a salt of ammonia are replaced by NH_4. If, on the other hand, the equation is NH_4—zeolite, the opposite is the case. In order to test the theory, the following experiment was made.

Fifty grams of soil II containing 5.04 mgm. ammonia-nitrogen in accordance with the Boussingault method, were admixed with 300 cc. of $(NH_4)_2SO_4$ solution (53.76 mgm. N). After 48 hours the solution was filtered and washed with 400 cc. distilled water, and the ammonia-nitrogen of the filtrate was determined at 31.29 mgm. The residue of the filtrate was then allowed to stand with 300 cc. $MgCl_2$ solution (1 gm. $MgCl_2+6H_2O$), whereupon it was filtered and washed with distilled water. This latter filtrate contained 9.03 mgm. ammonia nitrogen and in the undissolved residue 12.32 mgm. was found. The nitrogen loss was consequently 11.46 per cent of the amount of nitrogen added. In a corresponding parallel determination by the Boussingault method (distillation of 50 gm. soil + 53.76 mgm. ammonia-nitrogen after 48 hours) the loss was 7.42 per cent. In this instance, therefore, nothing had been gained by the substitution process.

It is thus manifest from all our experiments that there exists at present no method which permits of an accurate quantitative extraction of ammonia from any soil. Thus in the Boussingault method, which was employed by us in all the following experiments, it will always be necessary to reckon with some loss. However, in our experiments these losses are scarcely of any account, because the absolute losses in the soils worked with are relatively small, and also because the experiments in question are concerned with relative values.

EXPERIMENTS RELATING TO THE ACTION OF LIME ON THE NITRIFICATION OF STABLE-MANURE NITROGEN IN SOILS OF DIFFERENT REACTION

In the first experiment the soil designated above as soil II was used. This was taken from the experimental field of the Botanical Section of Experimental-fältet, and consisted of an originally stiff clay soil, now well manured, friable, and well tilled. The reaction to litmus was neutral. As the *Azotobacter* test gave a positive reaction, this soil was evidently not in need of lime. Before the soil was placed in the glass jars, it was riddled and supplied with the following carefully mixed ingredients.

Jar   I No admixture (control).
Jar   II 10 gm. $(NH_4)_2SO_4$ (0.2 per cent).
Jar   III 10 gm. $(NH_4)_2SO_4$ + 89.5 gm. $CaCO_3$ (1 per cent CaO).
Jar   IV 150 gm. 3 to 4 months old barnyard manure (3 per cent).[1]
Jar   V Manure + lime in the same quantities as before.

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[1] The content of total nitrogen and of ammonia-nitrogen in the manure was determined at each experiment, but these figures are not give here. In order to obtain clear results only manures whose content of ammonia-nitrogen exceeded 0.14 per cent were used.

The manure did not contain any liquid, as the latter had been collected separately in a special manure-water well.

Both the nitrate-nitrogen and the ammonia-nitrogen in this experiment, as in all the following, are expressed in terms of milligrams per kilogram of moist earth.

The results of this first experiment are shown in table 6. The content of moisture in the soil is not given in each separate case. During the entire course of the experiment it was about 20 per cent in the jars which did not contain any manure, and in the manure jars 21 to 22 per cent.

In examining these figures it should be borne in mind that one must not look for precise agreement even where theoretically it might be expected, as, for example, in the content of nitrate in all the jars at the beginning of the experiment, or the content of ammonia at the beginning in jars II and III, or IV and V, respectively. Such divergencies in work of this kind are inevitable, partly because of the impossibility of mixing with perfect homogeneity the components added to the soil, and partly because of the difficulty of obtaining actual average samples from the jars. In spite of such discrepancies, however, various conclusions may be drawn from the experiment.

First, the content of nitrate in the soil is unusually high from the very outset, so high that any greater nitrification in the control soil is scarcely noticeable. This can be explained, at any rate in part, by the fact that the experimental soil, being originally rather moist, had first to be spread out to dry at ordinary temperature before it could be put into the glass jars. During this drying, however, a very intense nitrification takes place.

It will further be found that the admixture of lime has an extremely stimulating effect on the nitrification of the ammonium sulfate. It is precisely for the purpose of establishing this effect that the series with ammonium sulfate and that with ammonium sulfate plus lime have been included in all our experiments.

The nitrification of the manure-nitrogen also was rather marked. In previous experiments (2) it has been proved that it is only the ammonia-nitrogen of the manure that is nitrified. This is confirmed also by the present and following experiments, which show that the nitrate-nitrogen formed does not exceed the ammonia-nitrogen present from the outset in the mixture of soil and manure, deducting the normal content of ammonia-nitrogen in the soil. The soil always contains a residue of 20 to 50 mgm. of ammonia-nitrogen per kilogram which does not allow of nitrification. This may conceivably be due to the fact that this nitrogen originally did not consist of ammonia-nitrogen proper, but was produced after distilling with MgO by the splitting-off of higher compounds of nitrogen.

Proceeding to the main objective of the experiments, namely, to the possible action of the lime on the nitrification of the stable-manure nitrogen, table 6 shows clearly that in this experiment no stimulating action of the lime can be found. On the contrary, the average of the nitrate-nitrogen for the entire series is lower for the manure plus lime than for the manure alone.

TABLE 6

Influence of lime on nitrification—First experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE-NITROGEN AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL											
			0 days	7 days	14 days	21 days	28 days	35 days	41 days	49 days	56 days	146 days	236 days	334 days
			mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	None	Nitrate-nitrogen	48.0	46.0	48.0	52.0	52.0	52.0	48.0	52.0	52.0	56.0	56.0	72.0
		Ammonia-nitrogen	47.6	44.8	44.8	44.8	47.6	42.0	—	44.8	36.4	25.2	50.4	39.2
II	0.2 per cent $(NH_4)_2SO_4$	Nitrate-nitrogen	32.0	68.0	96.0	170.0	210.0	280.0	200.0	220.0	200.0	240.0	290.0	100.0
		Ammonia-nitrogen	414.4	411.6	310.8	252.0	240.8	196.0	140.0	120.4	95.2	28.0	42.0	42.0
III	0.2 per cent $(NH_4)_2SO_4$ + 1 per cent CaO	Nitrate-nitrogen	28.0	60.0	128.0	200.0	260.0	280.0	340.0	360.0	280.0	290.0	280.0	340.0
		Ammonia-nitrogen	456.4	436.8	386.4	285.6	201.6	114.8	44.8	42.0	36.4	28.0	—	42.0
IV	3 per cent manure	Nitrate-nitrogen	40.0	60.0	56.0	68.0	76.0	68.0	80.0	80.0	80.0	100.0	88.0	96.0
		Ammonia-nitrogen	84.0	47.6	44.8	47.6	50.4	50.4	42.0	50.4	39.2	28.0	47.6	42.0
V	3 per cent manure + 1 ... CaO	Nitrate-nitrogen	44.0	60.0	58.0	80.0	76.0	72.0	60.0	68.0	72.0	68.0	76.0	88.0
		Ammonia-nitrogen	70.0	47.6	50.4	47.6	53.2	50.4	42.0	47.6	42.0	28.0	44.8	47.6

Stephenson (10), who examined the effect of organic matter on the produc-
ion of nitrate and ammonia nitrogen in different soils, in this connection ob-
ained similar results. He says that "the sum of the nitrates and ammonia is
reater in most cases on the unlimed than on the limed pots where organic
reatments are given."

In the next experiment the proportion of lime to manure was increased, the
atio being 2 per cent of CaO to 1 per cent of manure, as compared with 1
nd 3 in the preceding experiment. In other respects, both the experimental
oil and the amount of ammonium sulfate added were the same as before.
The water content of the soil was about 22 per cent. The results of this
xperiment are shown by table 7.

In view of the greatly reduced admixture of manure in this experiment, one
ould hardly expect to obtain very high nitrate figures for jar IV. It is, how-
ver, possible to show a more powerful nitrification here than in the control
ir. The admixture of lime in this experiment also gave a negative result,
ince the amount of nitrate in jar V is somewhat lower than in jar IV, and as
matter of fact does not exceed that of the control jar. Here it seems to be
ractically certain that the addition of lime impeded the nitrification of the
itrogen of the barnyard-manure.

In this experiment and those that follow, a control series was conducted
rith the same soil and the same admixtures, but at a different time. The con-
rol series naturally did not give the same absolute figures for the nitrate and
he ammonia nitrogen as the corresponding series, but the relative proportions
rere invariably the same. The results of the control series are not given here,
s this would unnecessarily congest the tabular material, but the fact that they
rere always carried out, and invariably agreed well with the experiments de-
cribed is strong proof of the reliability of the results.

In the succeeding experiment the same amount of lime was retained, but
he supply of manure was increased to 5 per cent. Such an amount of manure,
f course, is much too large for practical purposes, being equivalent to no less
han 150,000 kgm. per hectare, while the amounts previously used are equiva-
ent to 90,000 and 30,000 kgm. per hectare. However, in these experiments the
dmixtures were greatly varied because the aim was to ascertain whether the
elative amounts of lime and manure had any influence at all on the results.
The soil used in this experiment was the one designated as soil I in the ammonia
xperiments. It is a well-cultivated clay loam (ancient sea bottom) from
Experimentalfältet. The reaction was neutral and the *Azotobacter* test gave a
ositive result. The soil has long been in a high state of fertility.

From table 8 it can be seen that here also the lime considerably impeded the
itrification of the stable-manure nitrogen. The original content of nitrate in
he soil was very low. The ammonium sulfate added was not nitrified as
nuch as in the preceding experiments, but when lime was added, this
itrification, on the other hand, was much greater than in the experiments
with soil II. It will be found in this, as in preceding experiments, that in

TABLE 7

Influence of lime on nitrification—Second experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL								
			0 days	14 days	21 days	28 days	35 days	42 days	138 days	264 days	356 days
			mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	None	Nitrate-nitrogen	32.0	38.0	36.0	36.0	39.0	48.0	56.0	52.0	44.0
		Ammonia-nitrogen	56.0	50.4	64.4	58.8	61.6	56.0	33.6	44.8	47.6
II	0.2 per cent (NH₄)₂SO₄	Nitrate-nitrogen	26.0	112.0	200.0	220.0	240.0	300.0	360.0	230.0	220.0
		Ammonia-nitrogen	425.6	291.2	263.2	—	207.2	154.0	39.2	44.8	39.2
III	0.2 per cent (NH₄)₂SO₄ + 2 per cent CaO	Nitrate-nitrogen	-20.0	180.0	260.0	280.0	300.0	380.0	360.0	270.0	200.0
		Ammonia-nitrogen	383.6	170.8	64.4	56.0	53.2	53.2	36.4	44.8	42.0
IV	1 per cent manure	Nitrate-nitrogen	28.0	36.0	36.0	40.0	46.0	56.0	68.0	64.0	56.0
		Ammonia-nitrogen	56.0	58.8	44.8	58.8	53.2	56.2	36.4	50.4	53.2
V	1 per cent manure + 2 per cent CaO	Nitrate-nitrogen	26.0	36.0	36.0	36.0	40.0	48.0	56.0	56.0	60.0
		Ammonia-nitrogen	53.2	53.2	47.6	61.6	56.0	50.4	39.2	50.4	53.2

TABLE 8

Influence of lime on nitrification—Third experiment

LAB. NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL													
			0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days	77 days	84 days	91 days
			mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	None	Nitrate-nitrogen	1.0	3.6	4.0	5.5	6.0	8.0	8.0	8.0	8.0	10.0	8.0	10.0	12.0	10.0
		Ammonia-nitrogen	33.6	30.8	22.4	25.2	33.6	22.4	28.0	33.6	39.2	25.2	19.6	19.6	25.2	19.6
II	0.2 per cent (NH₄)₂SO₄	Nitrate-nitrogen	0.8	5.5	15.0	24.0	44.0	68.0	65.0	65.0	70.0	70.0	80.0	90.0	80.0	70.0
		Ammonia-nitrogen	—	476.0	434.0	361.2	324.8	319.2	302.4	285.4	260.4	246.4	240.8	243.6	251.2	232.4
III	0.2 per cent (NH₄)₂SO₄ + 2 per cent CaO	Nitrate-nitrogen	1.0	11.0	48.0	80.0	180.0	220.0	260.0	240.0	220.0	240.0	200.0	220.0	200.0	140.0
		Ammonia-nitrogen	504.0	420.0	252.8	170.8	33.6	25.2	28.0	22.4	30.8	19.6	19.6	16.8	28.0	25.2
IV	5 per cent manure	Nitrate-nitrogen	1.6	10.0	16.0	20.0	28.0	30.0	30.0	44.0	32.0	36.0	28.0	34.0	28.0	18.0
		Ammonia-nitrogen	70.0	56.0	28.0	39.2	28.0	28.0	16.8	28.0	28.0	33.6	28.0	22.4	39.2	22.4
V	5 per cent manure + 2 per cent CaO	Nitrate-nitrogen	1.2	8.0	7.5	14.0	16.0	20.0	18.0	12.0	14.0	18.0	16.0	16.0	16.0	14.0
		Ammonia-nitrogen	56.0	33.6	28.0	28.0	16.8	22.4	19.6	36.4	25.2	19.6	22.4	22.4	28.0	28.0

the case of the admixture of ammonium sulfate (especially with NH_4SO_4 plus CaO,, a continuous diminution occurs in the sum of the nitrate and ammonia nitrogen at each sampling from the different jars. This can be explained by an assimilation brought about by microorganisms. It seems hardly conceivable that ammonia losses should arise by a purely chemical process, i.e., by the action of the lime, if one may judge by the experiments made in particular by Lemmermann and his collaborators. On the other hand, it has been found that the addition of lime markedly promotes the assimilation of ammonia and nitrate in the soil. In the jars containing manure no such reduction of the summed-up nitrate and ammonia nitrogen occurs.

All the above experiments are open to the objection that they have been made with amounts of lime which greatly exceed those which are used in practice. It is, in fact, probable that when the amount of lime added has exceeded a certain limit, a further increase can have no further effect. Such large amounts of lime were here used in order to ensure the clearest possible results. However, in order to be certain that the conditions prevailing when normal amounts of lime are added are not different in principle from those present in the experiments, soil II was used for another experiment, in which lime was added at the rates of 0.2 and 0.1 per cent, being equivalent to 6,000 and 3,000 kgm. CaO per hectare. Manure was used at the rate of 2 per cent, being equivalent to 60,000 kgm. per hectare. The results obtained are given in table 9.

The figures of this table show clearly, as was to be expected, that the small amounts of lime had no greater stimulating effect on the nitrification of the stable manure than the larger. But, on the other hand, no "impeding" action by lime could here be established, as occurred with the larger applications, since nitrification evidently proceeded quite uniformly in jars IV, V, and VII.

All the experiments hitherto described have been made with neutral soils, which, according to the *Azotobacter* test, were not in need of lime. It might reasonably be argued that the cause of the negative result of the addition of lime to these soils in regard to its action on the nitrification of the manure nitrogen is simply the fact that the lime is unable to react on a soil which already contains, from a biological point of view, a sufficient amount of lime.

It is evident, nevertheless, that the lime had a certain action in our experiments, first, because the jars which, in addition to manure, contained larger quantities of lime, showed throughout an inferior nitrification to those which contained simply manure; and, second, because the lime, even in these soils which were not in need of lime, always showed a strong capacity for stimulating the nitrification of ammonium sulfate. This action is still quite evident with an addition of 0.2 per cent CaO, whereas it is hardly perceptible at 0.1 per cent.

It was conceivable that in manifestly acid soils the lime might have another effect on the nitrification of the manure-nitrogen than had been ascertained to be the case in the neutral soils hitherto used. Similar experiments were therefore conducted with an acid-reacting soil, a clay soil from Ensta in upland.

TABLE 9

Influence of lime on nitrification—Fourth experiment

NITRATE- AND AMMONIA-NITROGEN PER KILOGRAM OF SOIL

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days	63 days	70 days	77 days	84 days	98 days
			mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	None	Nitrate-nitrogen	5.5	6.0	7.5	8.0	8.5	9.0	10.0	10.5	11.0	11.0	10.5	12.0	12.0	12.5
		Ammonia-nitrogen	36.4	47.6	·42.0	39.2	30.8	47.6	50.4	44.8	39.2	22.4	47.6	47.6	36.4	39.2
II	0.2 per cent (NH$_4$)$_2$SO$_4$	Nitrate-nitrogen	5.0	30.0	65.0	100.0	100.0	110.0	130.0	160.0	180.0	190.0	210.0	210.0	220.0	220.0
		Ammonia-nitrogen	341.6	403.2	302.4	288.4	252.0	193.2	128.8	84.0	58.8	58.8	47.6	42.0	36.4	39.2
III	0.2 per cent (NH$_4$)$_2$SO$_4$ + 0.2 per cent CaO	Nitrate-nitrogen	5.5	28.0	70.0	105.0	110.0	150.0	190.0	180.0	140.0	190.0	230.0	220.0	220.0	210.0
		Ammonia-nitrogen	394.8	375.2	296.8	238.0	182.0	112.0	39.2	50.4	42.0	47.6	47.6	36.4	33.6	44.8
IV	2 per cent manure	Nitrate-nitrogen	6.0	11.5	15.0	14.0	15.0	15.0	17.0	17.0	16.0	17.0	17.0	21.0	21.0	22.0
		Ammonia-nitrogen	47.6	36.4	42.0	47.6	44.8	50.4	33.6	44.8	44.8	36.4	42.0	47.6	39.2	39.2
V	2 per cent manure + 0.2 per cent CaO	Nitrate-nitrogen	6.0	11.5	13.0	14.0	15.0	16.0	17.0	17.0	16.0	17.0	17.0	19.0	21.0	21.0
		Ammonia-nitrogen	50.4	39.2	50.4	42.0	44.8	50.4	33.6	47.6	47.6	42.0	44.8	42.0	44.8	42.0
VI	0.2 per cent (NH$_4$)$_2$SO$_4$ + 0.2 per cent CaO	Nitrate-nitrogen	5.0	23.0	60.0	80.0	85.0	130.0	150.0	170.0	160.0	200.0	210.0	210.0	230.0	230.0
		Ammonia-nitrogen	383.6	350.0	308.0	263.2	235.2	173.6	114.8	61.6	53.2	44.8	50.4	50.4	47.6	42.0
VII	2 per cent manure + 0.1 per cent CaO	Nitrate-nitrogen	6.5	12.0	15.0	14.0	15.0	15.0	18.0	17.0	17.0	14.0	17.0	20.0	21.0	21.0
		Ammonia-nitrogen	64.4	50.4	44.8	47.6	58.8	58.8	50.4	53.2	53.2	47.6	50.4	56.0	47.6	50.4
VIII	0.2 per cent CaO	Nitrate-nitrogen	5.0	5.0	7.0	8.0	8.0	8.5	9.5	9.0	9.0	8.0	9.5	10.5	10.5	12.0
		Ammonia-nitrogen	50.4	44.8	56.0	—	58.8	58.8	67.2	53.2	53.2	56.0	53.2	56.0	47.6	50.4

TABLE 10

Influence of lime on nitrification — Fifth experiment

JAR NUMBER	ADMIXTURE PER KILOGRAM OF SOIL		0 days	8 days	16 days	22 days	30 days	38 days	44 days	52 days	58 days	67 days	73 days	84 days	93 days	121 days
I	None	Nitrate nitrogen														
		Ammonia nitrogen														
II	0.2 per cent (NH₄)₂SO₄	Nitrate nitrogen														
		Ammonia nitrogen														
III	0.2 per cent (NH₄)₂SO₄ + 2 per cent CaO	Nitrate nitrogen														
		Ammonia nitrogen														
IV	4 per cent manure	Nitrate nitrogen														
		Ammonia nitrogen														
V	4 per cent manure + 2 per cent CaO	Nitrate nitrogen														
		Ammonia nitrogen														
VI	4 per cent manure + 0.2 per cent (NH₄)₂SO₄	Nitrate nitrogen														
		Ammonia nitrogen														
VII	4 per cent manure + 0.2 per cent (NH₄)₂SO₄ + 2 per cent CaO	Nitrate nitrogen														
		Ammonia nitrogen														
VIII	2 per cent CaO	Nitrate nitrogen														
		Ammonia nitrogen														

he same soil had previously been used in our experiments on the nitrification f stable manure in soils of different reaction.

This soil is in a high state of cultivation. In the qualitative test of its acid-y in accordance with the Truog method, it was shown to be of "medium :idity." The reaction to litmus was evidently acid. The *Azotobacter* test ıve a negative result.

In the execution of the experiment exactly the same procedure as in the fore-ɔing was adopted. The admixture of lime was 2 per cent and the amount of .able manure was 4 per cent of the weight of the soil. As with the preceding ils, two exactly similar series of experiments were made at different times. s in this case also the results of the two series agreed with one another very osely, the results of only one series are given (table 10).

The interesting facts shown here with regard to the nitrification in the Ensta il without admixture, in the soil with $(NH_4)_2SO_4$ alone, and in the soil with ıanure only, have been discussed in a previous paper (2) so are not taken up ere. It need only be pointed out that the acid reaction of the soil did not :and in the way of a strong nitrification, either in the soil without admixture, r in the soil with manure, whereas in the soil with $(NH_4)_2SO_4$ the latter was itrified very poorly, even more poorly than the stable-manure nitrogen. 'his fact, which has also been pointed out by other scientists, has been ex-lained in the above-mentioned paper as follows:

The increase in the concentration of hydrogen-ions which sets in when the ammonium ılfate begins to be decomposed in acid soils, has an impeding action on the nitrification, hereas in the case of the decomposition of the organic nitrogenous substances no such in-ease of the concentration of the hydrogen-ions need be considered.

As for the action of the lime, it is evident from the figures for jars IV and V hat the lime had a no more favorable action on the nitrification of the stable-ıanure nitrogen in this acid soil than in the neutral soils. On the contrary, manifest impeding effect is noticeable here. On the addition of lime alone ɔ the soil a slight increase in the nitrification is certainly noticeable, but not ntil after the lapse of three or four months. On the other hand, as was to be xpected, the lime had a decidedly favorable effect on the nitrification of the mmonium sulfate.

It should be pointed out that the quantity of lime added is far more than ufficient to neutralize the soil.

SUMMARY

All the experiments tended to show that on the addition of lime, in the form f calcium carbonate, and stable manure to cultivated soil of both neutral .nd acid reaction, no favorable action of the lime on the *nitrification of the stable-nanure nitrogen resulted*, but on the contrary, an impeding effect, occurred in :ases where the supply of lime was very large, larger than is probably ever ısed in practice.

It is not easy to explain the cause of this impeding action of the lime. Fred and Graul (5), who made experiments on the action of lime on the nitrification of organic substances in acid soils, found, that in the presence of casein or gelatin the addition of lime produced an enormous increase of nitrate-assimilating bacteria, and it is quite possible that the same will be the case if, as in our experiments, stable manure and lime are simultaneously supplied to the soil. If this is really the case, this impeding of nitrification does not imply any loss of nitrogen, absolutely speaking, since the nitrogen which the nitrate-assimilating baceria extracted is stored in the form of bacterial proteins and is sooner or later utilized on the death of the bacterial cells.

If, on the other hand, the impeding action of the lime on the nitrification of the stable-manure nitrogen is due to an increased development of denitrifying bacteria, a direct loss of nitrogen is naturally conceivable. At any rate these questions appear to be of no practical importance, for, as has already been pointed out, the lime does not exercise either an impeding or a stimulating action when it is supplied in quantities which occur in practice.

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QUANTITATIVE AND QUALITATIVE BACTERIAL ANALYSIS OF SOIL SAMPLES TAKEN IN FALL OF 1918

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A bacterial analysis was made of 46 soils representing 17 soil types obtained in a soil survey of Russell, Carleton and Stormont counties in Eastern Ontario. Each soil was represented by two samples, one taken from the surface to a depth of 6⅔ inches, and the other a sub-surface sample taken from 6⅔ to 20 inches depth. The lower subsoils were not examined for bacterial content. These soils were filled directly into cotton bags in the field where obtained and shipped to the college by freight. On arrival at the chemistry department they were emptied into large open-topped dishes for air-drying in the attic, which was clean and free from dust. The samples for bacterial analysis were taken from these open dishes a few days after their arrival and put directly into clean, dry 500-cc. Erlenmeyer flasks which were half filled and then corked and kept at a low temperature in the attic of the bacteriology department until December 15, when the analyses were begun.

The purpose of the analysis was to determine the relative bacterial and mold content of the different soil samples. Three culture media were used as follows:

1. *Albumen Synthetic Agar* (A) (P. E. Brown) for obtaining the total bacterial and mold counts.

2. *Nutrient Gelatin* for obtaining the liquefier counts.

3. *Modified Ashby's Agar*[1] for obtaining the *Azotobacter, Ps. radicicola* and *Nocardia* counts.

SOIL DILUTIONS

After thoroughly mixing the soil samples and discarding any gravel or lumps, 20 gm. of the remaining soil were weighed out on a piece of flamed copperfoil, and put into a 500-cc. Erlenmeyer flask containing 200 cc. of sterile water.

This was shaken for 5 minutes and after the coarser particles had settled, 10 cc. of the mixture was transferred to a 90-cc. sterile water blank which was thoroughly shaken, and from this 10 cc. was transferred to another 90-cc. water blank and further dilutions were made in the same way until a series of 1/10, 1/100, 1/1,000, 1/10,000, 1/100,000 and 1/1,000,000 gram of soil

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[1] Ashby's agar was modified by using 10 gm. of cane sugar in place of 20 gm. of mannite.

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per cubic centimeter were obtained. In general the higher dilutions were used for the total counts of the surface soils, and the lower dilutions for the subsoils.

The prevailing types of colonies were isolated from the various plate cultures on the tubes of similar medium to that of the plate from which they were taken. These tube cultures were then used for making microscopic preparations for describing the organisms.

The counts recorded are the average counts from plate cultures made from three dilutions of each soil sample.

The *Ps. radicicola* counts are only approximate, as the colony appearance of some other species of bacteria rather closely resembles that of *Ps. radicicola*.

TABLE 1

Soil type I—Yellow sand

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	1	Typical.	1,373,000	413,000	0	0	20,000	120,000
Sub.	2		433,000	403,000	0	0	30,000	65,000
Sur.	4	Finer sand, simi-	380,000	10,000	0	0	100,000	140,000
Sub.	5	lar to type 2	340,000	2,000	0	0	400,000	65,000

TABLE 2

Soil type 2—Light to medium brown, fine sand and sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	7	Representing the	910,000	56,000	0	0	300,000	190,000
Sub.	8	fine sand. Clay subsoil	950,000	1,000	0	0	100,000	100,000
Sur.	10	Same field as 7-8	273,000	24,000	0	0	200,000	5,500
Sub.	11	but taken from hollows	200,000	2,000	0	0	0	10,000
Sur.	13	Shallow, not so	740,000	77,000	0	100,000	300,000	500,000
Sub.	14	sandy. Clay subsoil of 13	1,300,000	5,000	0	100,000	200,000	130,000
Sur.	17	Representing the	810,000	46,000	0	200,000	600,000	80,000
Sub.	18	fine sandy loam	270,000	8,000	0	0	0	60,000
Sur.	20	Typical. Same as	140,000	19,000	0	70,000	240,000	10,000
Sub.	21	17-18	433,000	8,000	0	30,000	80,000	25,000
Sur.	23	Same as 13-14	2,150,000	100,000	0	0	200,000	670,00
Sub.	24	but clay sub-soil of 8	1,880,000	15,000	0	400,000	0	100,00

TABLE 3

Soil type 3—Light to medium brown, gravelly, sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	26	Typical	2,650,000	105,000	195	900,000	600,000	20,000
Sub.	27		3,350,000	27,000	75	1,200,000	800,000	185,000
Sur.	29	Flat, sand	2,270,000	240,000	40	500,000	200,000	130,000
Sub.	30		1,110,000	38,000	20	700,000	200,000	60,000
Sur.	32	Typical	1,750,000	91,000	0	200,000	100,000	1,200,000
Sub.	33		1,000,000	54,000	0	200,000	100,000	470,000
Sur.	35	Typical	1,440,000	41,000	10	200,000	400,000	260,000
Sub.	36		650,000	13,000	0	0	200,000	320,000

TABLE 4

Soil type 4—Medium brown loam, in which stones vary in size and quantity. Variable gravel content

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	38	Typical of me-	1,730,000	12,000	1,710	600,000	1,400,000	40,000
Sub.	39	dium soil	1,110,000	55,000	40	100,000	200,000	100,000
Sur.	41	Less stone and	3,780,000	30,500	230	1,000,000	700,000	0
Sub.	42	gravel in sur-face, more clay in subsoil	2,980,000	210,000	0	200,000	600,000	10,000
Sur.	44	Probably belongs	950,000	355,000	0	100,000	200,000	0
Sub.	45	to type 3	1,020,000	25,500	0	600,000	300,000	0
Sur.	47	Shallow soil,	1,030,000	64,500	170	400,000	800,000	30,000
Sub.	48	hard-pan or boulder, clay subsoil	513,000	64,500	120	0	100,000	80,000
Sur.	49	About the same	3,270,000	66,000	520	400,000	300,000	200,000
Sub.	50	as 38–39	1,700,000	79,000	80	1,200,000	200,000	240,000
Sur.	52	Slightly more	3,300,000	155,000	345	400,000	900,000	550,000
Sub.	53	gravel than 49–50 and 38–39	2,330,000	95,000	70	300,000	200,000	295,000

TABLE 5
Soil type 5—Black sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI- ERS	AZOTOBAC- TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	55	Typical	3,970,000	260,000	410	200,000	1,300,000	140,000
Sub.	56		255,000	125,000	25	800,000	200,000	5,000
Sur.	58	Lighter in color	4,770,000	93,000	210	400,000	200,000	340,000
Sub.	59	than typical soil	610,000	55,000	0	200,000	0	55,000
Sur.	61	Typical	1,610,000	63,000	10	0	400,000	65,000
Sub.	62		1,320,000	8,500	10	200,000	0	22,000
Sur.	64	Same area as 58–	2,230,000	53,000	0	0	100,000	21,500
Sub.	65	59, practically belongs to type 2	540,000	8,500	0	0	0	65,000

TABLE 6
Soil type 6—Sandy clay loam, usually dark brown

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI- ERS	AZOTOBAC- TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	67	Typical except	2,870,000	61,000	660	1,900,000	300,000	10,000
Sub.	68	that the sand is of finer grade than usual	1,830,000	55,000	80	1,600,000	0	0
Sur.	70	Probably belongs	4,100,000	160,000	85	800,000	400,000	40,000
Sub.	71	to type 8	650,000	15,500	0	400,000	0	20,000
Sur.	73	Typical	3,750,000	190,000	1,800	T.M.	T.M.	0
Sub.	74		3,250,000	185,000	125	T.M.	T.M.	0

TABLE 7
Soil type 7—Brown, and usually moderately stony, clay loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI- ERS	AZOTOBAC- TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	76	Lighter than typ-	3,700,000	170,000	970	700,000	1,000,000	170,000
Sub.	77	ical soil	2,500,000	226,000	850	800,000	600,000	75,000
Sur.	79	Heavier than	9,100,000	68,000	60	5,000,000	700,000	230,000
Sub.	80	typical soil	4,600,000	67,000	0	3,500,000	200,000	260,000
Sur.	82	Typical	4,400,000	750,000	120	1,000,000	1,500,000	20,000
Sub.	83		3,400,000	900,000	75	600,000	850,000	15,000
Sur.	85	Typical	8,500,000	122,000	0	500,000	1,900,000	245,000
Sub.	86		1,670,000	4,000	0	1,000,000	100,000	210,000

TABLE 8

Soil type 8—Very fine, sandy, heavy clay loam and clay, usually dark gray to black

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	88	Typical	6,980,000	385,000	90	1,500,000	1,200,000	195,000
Sub.	89		666,000	20,500	0	400,000	100,000	65,000
Sur.	91	Surface a little	2,680,000	40,000	0	200,000	100,000	20,000
Sub.	92	lighter than 88	1,020,000	20,500	0	300,000	0	0
		−89						
Sur.	94	Typical, but not	5,100,000	76,000	0	1,400,000	700,000	135,000
Sub.	95	so old; higher	690,000	4,000	0	0	0	30,000
		nitrogen con-						
		tent						

TABLE 9

Soil type 9—Muck and peat with clay subsoil

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	97	Typical	5,270,000	850,000	0	700,000	1,900,000	75,000
Sub.	98		880,000	28,500	0	90,000	70,000	200,000

TABLE 10

Soil type 10—Very fine, sandy, silty loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	100		1,700,000	47,000	230	500,000	0	185,000
Sub.	101		155,000	12,000	0	300,000	100,000	150,000
Sur.	103	Typical	2,300,000	650,000	770	480,000	620,000	25,000
Sub.	104		1,060,000	390,000	200	960,000	350,000	30,000

TABLE 11

Soil type 11—Silty loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	106	Typical	385,000	280,000	60	120,000	350,000	260,000
Sub.	107		640,000	60,500	0	220,000	200,000	75,000

TABLE 12

Soil type 12—Red shale loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	112	Typical	3,150,000	585,000	0	270,000	830,000	75,000
Sub.	113		3,900,000	250,000	0	450,000	T.M.	95,000

TABLE 13

Soil type 13—Brownish red clay (shale)

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTO-BACTER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	114	An average sam-ple of the large areas belonging to this type, though color not quite typi-cal	1,780,000	26,500	0	300,000	700,000	30,000
Sub.	115		1,720,000	9,000	0	0	700,000	80,000
Sur.	117	Typical	9,800,000	155,000	0	T.M.	T.M.	250,000
Sub.	118		14,400,000	59,000	0	T.M.	T.M.	1,000

TABLE 14

Soil type 14—Like type 5 in texture, but belongs to a shale series

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	120	Typical	4,200,000	105,000	0	700,000	T.M.	0
Sub.	121		5,300,000	280,000	0	800,000	T.M.	0

TABLE 15

Soil type 15—Dark gray to black, shale clay

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	123	Typical	2,100,000	180,000	0	380,000	640,000	150,000
Sub.	124		4,100,000	38,000	0	160,000	730,000	10,000
Sur.	126	Also typical, but from an area that has not been cultivated so long as 123–124; more or-ganic matter	4,180,000	93,000	0	0	700,000	230,000
Sub.	127		1,730,000	10,500	0	0	200,000	190,000

TABLE 16

Soil type 16—Gray shale loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	129	A brown shale loam has more organic matter and is a good soil	4,830,000	435,000	0	1,000,000	800,000	120,000
Sub.	130		750,000	83,000	0	0	0	180,000
Sur.	132	Typical	3,000,000	57,000	0	580,000	400,000	200,000
Sub.	133		1,870,000	12,000	0	620,000	240,000	240,000

TABLE 17

Soil type 17—Stony, fine sandy loam

SAMPLE NUMBER		DEVIATIONS FROM TYPE	TOTAL BACTERIA COUNT	LIQUEFI-ERS	AZOTOBAC-TER	PS. RADICICOLA	NOCARDIA	MOLDS
Sur.	135	Typical of best phase; incon-veniently stony but passable as a soil	7,400,000	425,000	970	150,000	350,000	270,000
Sub.	136		4,200,000	50,000	295	420,000	190,000	320,000
Sur.	138	Bouldery and stony, only fit for pasture for sheep	3,500,000	47,000	0	900,000	600,000	315,000
Sub.	139		2,100,000	100,000	0	300,000	300,000	120,000

DISCUSSION

Relation of Azotobacter, the principal nitrogen-fixing species of soil bacteria, to the soil types

It will be seen from the above counts that *Azotobacter* were found present in 9 out of the 17 soil types examined and in 22 out of the 29 soil samples that represented these 9 types. These types included all the loams except the shale loam as follows:

SOIL TYPE NUMBER	SOIL TYPE	PRESENCE OF AZOTOBACTER
3	Light brown gravelly sandy loam	3 out of 4 samples gave Azotobacter
4	Medium brown loam, stone varying in size and quantity	5 out of 6 samples gave Azotobacter
5	Black sandy loam, clay subsoil	3 out of 4 samples gave Azotobacter
6	Sandy clay loam	3 out of 3 samples gave Azotobacter
7	Brown, moderately strong clay loam	3 out of 4 samples gave Azotobacter
8	Fine sandy heavy clay loam	1 out of 3 samples gave Azotobacter
10	Fine sandy silty loam	1 out of 3 samples gave Azotobacter
11	Silty loam	1 out of 1 sample gave Azotobacter
17	Stony fine sandy loam	1 out of 2 samples gave Azotobacter

On the other hand, no *Azotobacter* were found in the remaining 8 soil types which included the sand, peat-muck and shale soils as follows:

SOIL TYPE NUMBER	SOIL TYPE	ABSENCE OF AZOTOBACTER
1	Yellow sand	2 samples
2	Light brown sand and sandy loam	6 samples
9	Muck and peat with clay subsoil	1 sample
12	Red shale loam	1 sample
13	Brownish red clay shale	2 samples
14	Brownish red clay shale, but sandy	1 sample
15	Dark gray to black shale clay	2 samples
16	Gray shale loam	2 samples

We assume from the above that in all probability the light sandy soils had not sufficient organic matter present and the peat-muck and shale soils were too strongly acid to favor the development of the *Azotobacter* nitrogen-fixing bacteria.

Relation of Ps. radicicola, the legume root nodule bacteria, to the soil types

As the colony appearance of *Ps. radicicola* is rather closely simulated by several other species of bacteria, we cannot be sure that all the counts above recorded as *radicicola* are true members of this species, although every care was taken to discard any that were not true to type.

It is interesting to note that every soil type examined, with the exception of one only, i.e., type 1, yellow sand, had a fairly high *Ps. radicicola* content, and further, that in most cases the sub-surface samples had a higher *radicicola* content than the surface samples. This is as one might expect, as the *Ps. radicicola* would come largely from the decayed legume root nodules.

In general, the soils that were favorable for *Azotobacter* had the higher *radicicola* counts, but the fact that fairly high *radicicola* counts were obtained in the peat-muck and shale samples, which were presumably too acid for *Azotobacter* would indicate that some varieties of *Ps. radicicola* at least had a fair degree of toleration for acid soils.

We could not determine from a bacterial culture examination what different varieties of *Ps. radicicola* were present, as the different varieties are determined by the different 'varieties of legume with which they will associate.

Therefore, we cannot say whether or not all varieties of legumes could be grown satisfactorily on all these different soils, even though *Ps. radicicola* was found present in considerable numbers, as these numbers may represent only a limited number of legume varieties, thus limiting the choice of legumes to those varieties with which the varieties of *Ps. radicicola* found would associate.

Relation of the total bacterial count and liquefier count to the soil types

In general the total bacterial count and liquefier count represent the majority of bacterial species that are active in digesting and preparing the crude plant-food present in the soil, thereby rendering it available in a suitable condition for assimilation by the growing crops. The number found is considered in a general way as being indicative of soil fertility, as their action is essential in the preparation of the plant-food in the soil.

With the exception of sample 1, yellow sand, sample 2, light brown sand, and sample 11, silty loam, each of which has a very low total count, all the samples have a fair bacterial content for arable soils. It is interesting to note that the shales have as high a total count as the loams; as a matter of fact one shale, no. 13, has the highest count of all. This would indicate that while the shale and peat muck soils examined were not suitable for the nitrogen-fixing *Azotobacter*, they were about equally suitable with the loams for the species of the plant-food digesting bacteria.

Relation of Nocardia (Actinomycetes) count to the soil types

It is considered (J. Conn) that the Nocardia (Actinomycetes) or thread bacteria, found in soil are largely active in bringing about the decay of dead root fibers, particularly those of sod, thereby aiding in preparing them for food of growing crops.

The counts above recorded show that they were least numerous in the sand but averaged up much alike in the loams, peat muck and shales, in all of which they were fairly numerous.

Molds

It is not at present considered that molds have any important rôle to play in connection with soil fertility. It is, however, interesting to note from the above counts that type 1, the yellow sand which gave no Azotobacter, no radicicola and the lowest of all total bacterial counts, gave a fairly high mold count, while type 6, sandy clay loam, which gave the highest Azotobacter count, a very high radicicola and total bacteria count, showed no molds. With one other exception, type 14, a sandy clay shale, which also gave no molds, the mold content of the samples was fairly uniform.

A chemical and physical analysis of these soils is being made, respectively, in the chemistry and physics departments of the college.

STATEMENT OF THE OWNERSHIP, MANAGEMENT, CIRCULATION, ETC.
REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912,

Of Soil Science, published monthly at Baltimore, Maryland, for October 1, 1919.
State of New Jersey
County of Middlesex }ss.

Before me, a Notary Public in and for the State and county aforesaid, personally appeared
Carl R. Woodward, who, having been duly sworn according to law, deposes and says that he
is the Assistant Editor of Soil Science and that the following is, to the best of his knowledge
and belief a true statement of the ownership, management (and if a daily paper, the circula-
tion), etc., of the aforesaid publication for the date shown in the above caption, required by
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CARL R. WOODWARD, *Assistant Editor.*

Sworn to and subscribed before me this 19th day of September, 1919.

RUSSELL E. LONG, *Notary Public.*

[SEAL]   My commission expires January 31, 1924.

POTASSIUM-BEARING MINERALS AS A SOURCE OF POTASSIUM FOR PLANT GROWTH

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I. INTRODUCTION

The necessity of using potassium fertilizers in general agricultural practice is not so vital as the public has been led to believe. The fact that applications of potassium salts have caused increases in crop yields at many of the experiment stations of the United States, together with the propaganda work of the German Kali Syndicate, has led to the popular belief that potassium fertilization is essential for the most profitable crop production on most of our common agricultural soils. As a matter of fact, the majority of soils in the United States contain from 20,000 to more than 60,000 pounds (24) of potassium per acre—6$\frac{3}{4}$ inches (2,000,000 lbs.). While most of this is held in the relatively insoluble form of complex silicates, crop yields as a rule are not limited by deficiencies of available potassium. Furthermore, it is not probable that potassium would become a limiting element under soil treatments that would otherwise produce maximum yields, because those same treatments, such as returning organic manures, improving the physical condition of the soil, and the addition of the usual limiting elements, nitrogen and phosphorus, will tend toward the maintenance of an increasing amount of readily available potassium by the return of this element in residues and manure, by bringing about increased solution of mineral potassium and by increasing the root systems and hence the feeding power of the crops grown. The earth's crust, to a depth of ten miles, has been estimated to contan an average of 2.32 per cent of potassium (66). More recent calculations place the estimate at 2.46 per cent (33, p. 13). This indicates that the problem of potassium for agriculture is ultimately one of liberation rather than of supply for normal agricultural soils and the common farm crops.

Experiments at the Pennsylvania (33, p. 423, 428–431; 36), Ohio (33, p. 442), Illinois (33, p. 466; 34, 35) and other experiment stations indicate that potassium salts are applied, either alone or with other fertilizers, at a loss as a rule, and at best at a very small profit in a few instances.

While the above statements are true for the majority of our farm lands, there are quite large areas in Illinois and other states which are markedly deficient in potassium. These consist mainly of muck and deep peat soils, and light

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sandy soils. They contain from practically no potassium to 4000 pounds of potassium per acre—6$\frac{3}{4}$ inches. In the case of the sandy soils some are actually deficient in total potassium, while others, which respond equally well to potassium fertilization, contain considerable amounts of the element, but the most of it is contained in the coarser particles. Because of the relatively small surface of the sand particles, their potassium can not become available to plants to any appreciable extent. A sample of dune sand, representative of considerable areas in Illinois, was analyzed by the writer. Of the 20,600 pounds of potassium per 2,000,000 pounds which it contained, 94.93 per cent was contained in the combined sands and 5.10 per cent in the silt and clay combined.

On these peaty and sandy soils the use of potassium salts has proved quite profitable, and is in fact absolutely necessary to successful crop production on much of the peat soil. The prohibitive price of potassium fertilizers and their entire withdrawal from the market during the last four or five years has interfered seriously with the profitable cropping of these potassium-poor soils. Soluble potassium salts from any of the several American sources are too high-priced for agricultural use. Potassium-bearing minerals are very abundant in the United States, and are already being produced in commercial quantities in several states for other than agricultural purposes.

Potassium feldspars, orthoclase and microcline ($KAlSi_3O_8$) are extremely abundant throughout the United States. Deposits have been extensively developed, however, only in California and some of the eastern states, particularly Maine, Connecticut, Pennsylvania, New York and Maryland (15, 39). The average potassium content is 7 to 11 per cent and the average price per ton in 1914 was $8.31; in 1915, $8.33 and in 1916, $9.30 (15).

The Leucite Hills in Sweetwater County, Wyoming, are the principal source of leucite ($KAlSi_2O_6$) in the United States. The area has been carefully studied and mapped by Schultz and Cross (57) who estimate the minimum amount of commercially available potassium at 164,000,000 tons. The scarcity of leucite among igneous rocks is due to the fact that it is formed from the molten magma only when there is a deficiency of silica. Whenever sufficient silica is present orthoclase, which contains 50 per cent more molecules of SiO_2 than leucite, is formed (57, p. 13).

Alunite is a hydrated basic sulfate of potassium and aluminum ($K_2O.3Al_2O_3.4SO_3.6H_2O$). . . . . It is usually contaminated with silica and other impurities and often has a part of its potash replaced by soda. It is widely disseminated through porphyritic volcanic rocks as an alteration product of feldspars, but it usually occurs in such small quantity or is contaminated with so much gangue material that it has no commercial value (26, p. 429).

High-grade alunite is limited in its distribution mainly to certain sections of Utah, Nevada and Colorado. The deposits are easily worked, being near the surface, and according to estimates by Loughlin (45), contain enough recoverable potassium to supply a large part of the American demand. The

ease with which the potassium is completely extracted from alunite as the sulfate (ignition to 750°C. followed by leaching with water) will probably lead to its use almost exclusively for the production of commercial potassium sulfate.

Muscovite ($KH_2Al_3Si_3O_{12}$) is quite abundant in many parts of the United States and is produced commercially, principally as sheet mica, in twelve states. North Carolina produced in 1916, 63 per cent of the total output of the country; New Hampshire, 14 per cent and South Dakota 13 per cent. Scrap mica, being the clippings from the sheet mica trimming plants, is used, ground, in a number of industries and could be obtained for agricultural use should it prove profitable. The price of scrap mica up to 1913, for the 3000 to 4000 tons produced annually, was $11 to $13 a ton, and in 1916, $16 a ton (55).

Lepidolite is a complex micaceous silicate, in which part of the potassium is replaced by lithium. It is usually a mixture of the pure mineral and mica, and contains 8 to 9 per cent of potassium. It is produced in California, Maine and the Black Hills of South Dakota. It is used mainly as a source of lithium for the preparation of lithium salts, and probably would not be commercially available in large amounts for agricultural purposes. The present cost is $15 to $20 a ton (56).

The great drawback to the use of potassium minerals as fertilizers is their low solubility, and hence, comparative unavailability to plants. There have been many attempts to extract soluble potassium salts from minerals by various treatments, and also to subject the minerals to processes which would render the potassium readily available to plants, so that the treated minerals could be used directly upon the soil, thus avoiding the expensive processes of extraction and evaporation. The extent of these efforts is indicated by the fact that the United States Patent Office granted 128 patents for processes of treating silicate rocks for the extraction of potassium between 1904 and 1917 (26). It would seem that the difficulty of the low availability to plants of mineral potassium might be greatly lessened on peat soil, where the decomposition of the large amount of organic matter present, even though slow, should hasten the decomposition of the minerals.

The object of the experiments to be reported in this paper was (a) to determine to what extent the potassium in certain minerals will become available when mixed with peat soil, using both the growth of a crop and chemical analysis as a measure of the amount of soluble potassium, and (b) to determine the effect of certain more active organic materials and soluble salts upon the solubility of the potassium in those minerals.

II. REVIEW OF PREVIOUS INVESTIGATIONS

A complete review of all the work which has been done concerning the rendering available of soil potassium would obviously be outside the scope of this work. The idea of using powdered minerals, or "stone-meal" as a source of potassium is not new.

In 1848 Magnus (46) grew a barley crop to maturity, and obtained well matured grain in an artificial soil in which powdered feldspar was the sole source of potassium. Since then numerous experiments have been carried out in which the results recorded are more or less contradictory in regard to the potassium absorbed by the plants. The variations are no doubt due to the conditions of the experiments and in a still greater degree to the kinds of minerals, their source and stage of weathering.

Missoux in 1853 (48) noted the beneficial effect upon plant growth of waste rock dust from granite quarries.

H. Hoffman, 1861–1863 (32) conducted wheat culture tests for two years on plats 9 by 50 feet, using ground nephelin-dolerite containing 4.98 per cent of potassium and considerable amounts of calcium, magnesium and phosphorus. The crops on the fertilized plats had a much better appearance during June of each season, but the harvest showed larger yields of grain from the unfertilized plats.

During the next twenty years little was done with silicate minerals as fertilizers, though a great deal of work was reported dealing with their chemical decomposition and the nature of their weathering products.

In 1887 Aitken (1,1a) in Scotland grew peas and turnips on $\frac{1}{40}$-acre field plat treated with potassium sulfate and 120-mesh feldspar in comparison. The following yields were obtained from the treatments indicated:

TREATMENT	YIELD OF PEAS	YIELD OF TURNIPS
	pounds	*pounds*
No potassium	96	476
K_2SO_4, 3 pounds	114	482
Feldspar, 12 pounds	102	496

While the feldspar here produced results for peas almost equal to the soluble potassium salt, and a greater yield for turnips, the small percentage increases of both over the yields of the untreated checks, indicate that the soil was not particularly deficient in potassium. Furthermore, the work at the Rothamsted Station indicates that turnips do not respond readily to potassium fertilization. The average turnip yields on the Agdell field were increased by over 13,000 pounds by the use of phosphorus, while a further addition of mineral fertilizers consisting of sodium, potassium and magnesium salts produced no further increase (33, p. 346). On the Barn field, phosphorus and potassium produced no greater increases than phosphorus alone (33, p. 399).

Nilson (50) grew oats on a peat soil in Sweden, comparing feldspar with potassium sulfate, applying 2.5 to 4 times as much potassium in the feldspar as in the soluble salt. Phosphorus and nitrogen were supplied, the latter as crude Chile saltpeter. The yields were as follows, from plats of 100 plants each:

TREATMENT	YIELD OF OATS
	gm.
Nothing	17.3
P + K₂SO₄	394.1
P + Feldspar	328.0
P + N	439.5
P + N + K₂SO₄	603.5
P + N + Feldspar	463.7

These results indicate large increases where feldspar was used and still larger for K₂SO₄, but the experiment does not prove definitely that the increase is caused by the potassium in the mineral. The large yield from phosphorus and nitrogen would indicate either that the soil was not deficient in available potassium, or that potassium was being added in the fertilizer, probably as an impurity in the Chile saltpeter. The high yields from all the plats receiving phosphorus, together with the absence of a plat receiving phosphorus alone, suggests that phosphorus may be the limiting element in that soil and for that crop.

In 1889 Feilitzen (20, 21) grew crops on peat soils, and peat mixed with sand in sunken zinc cylinders, comparing feldspar with kainit as a source of potassium. With oats, equal yields were obtained with feldspar and kainit, but in later experiments, with clover, potatoes and peas, the feldspar jars produced no greater yields than where no feldspar was used. These results as a whole are decidedly unfavorable to the feldspar.

In the same year Ballentine (3) at the Maine Experiment Station reported results of pot experiments with feldspar, in which oats was grown in pure quartz sand. The relative yields from KCl, feldspar and no potassium were 100, 79 and 0, respectively. The author's conclusion was that oats could obtain sufficient potassium from the feldspar used to produce a heavy crop of grain, though the feldspar was inferior to soluble potassium salts.

Headden (29) grew oats to maturity in pure quartz sand and feldspar mixed, the plant-food elements other than potassium being supplied in available form. The feldspar was crushed to 1 mm. and included all the fine material. The crop removed 1.20 gm. K per jar in addition to that contained in the seeds. No comparison was made with other forms of potassium.

The earlier work (1901–1903) of Prianischnikov (52), in which crops of tobacco, buckwheat, flax, peas and millet were grown in acid-washed sand, indicated no benefit from orthoclase and but small benefit from mica. The use of ammonium salts as a source of nitrogen produced much smaller yields than sodium nitrate. The ammonium salts either "exerted no solvent action upon the potassium, or any benefit from such solution was overbalanced by the deleterious effect of acidity produced by the nitrification of the ammonium salts." Somewhat later the same investigator (53) carried out very carefully a series of well-planned experiments upon the sand and peat soils of northern

Russia. The object of the first of these was to determine whether plants can obtain potassium only from secondary minerals in the soil, roughly classed as zeolites, or also from the unweathered primary minerals. Nephelin rock, characterized by easy weathering and a tendency to form zeolites, and containing some zeolites was compared with muscovite and orthoclase in acid-washed sand. Hellriegel's nutrient solution, minus potassium, was supplied to all the jars. The results are reproduced in full:

Yields

	KCl	NEPHELIN	MUSCOVITE	ORTHOCLASE	NO K
	gm.	gm.	gm.	gm.	gm.
Mustard	13.95	12.68	11.20	5.88	2.77
Buckwheat	10.10	14.30	10.90	2.80	4.40
Millet	6.90	9.70	7.60	4.40	4.70

Percentage utilisation of the potassium supplied in the mineral

	NEPHELIN	MUSCOVITE
	per cent	per cent
Mustard	25	22
Buckwheat	38	30
Millet	23	17

Orthoclase here seems to be practically worthless while both nephelin and muscovite are approximately equal to soluble potassium in crop-producing power. The amount of potassium which these last two minerals give up to the crop is exceptionally large. A second experiment involved the use of ten different minerals with five crops. Samples of the minerals were also extracted by shaking with 10 per cent solutions of ammonium chloride and of barium chloride, both with and without boiling. The relative amounts of potassium obtained by these extractions were in harmony with the results of the plant culture experiments. The minerals used were finally classified as a result of these experiments into the following three groups, arranged in order of decreasing availability of the potassium:

  1. Nephelin.
     Micas, especially biotite.
  2. Phillipsite (a zeolite).
     Muscovite.
  3. Eläolith,
     Leucite,
     Apophyllite,
     Sanidin,
     Orthoclase,
     Microcline.

Wotschal (69) reported mica superior to the feldspars.

The investigations of Cushman (15) are not conclusive, because the soils used in greenhouse work as well as in cooperative field tests by farmers, were probably not deficient in potassium, most of them having been heavily "potashed" for tobacco the previous years. In these tests, however, the yields of tobacco were the same whether potassium was supplied in feldspar or in potassium carbonate.

Hartwell and Pember (28) secured increases in yields of wheat and millet of 4 to 18 per cent for feldspar as compared with 130 to 248 per cent increases for potassium sulfate. The soil used had been cropped several years with liberal applications of the other plant-food elements, but no potassium. Fifteen pounds of soil were used in each jar.

Skinner and Jackson (58) increased crop yields by 10 to 20 per cent by applications of raw alunite. The same amounts (25 to 500 pounds K_2O per acre) of potassium supplied in ignited alunite produced increases of 35 to 43 per cent.

It is a well-known fact that the calcium and sodium feldspars are much more easily weathered than those of potassium. In the sodium-potassium feldspars, the rapidity of weathering should therefore increase with the proportion of sodium present. In 1913 Blanck (8) reported results of an investigation of the availability of the potassium of a series of feldspars decreasing progressively in potassium content, and increasing in sodium. The minerals were applied so that all jars received equal amounts of potassium, and, consequently, increasing amounts of sodium. Crop yields were increased progressively, in accordance with the hypothesis. This led the investigator to suspect that sodium was replacing potassium in its function in the plant. Analysis of the crops, however, proved that the increases in yields were due to potassium. Following is a summary of the results:

	K IN MINERAL	Na IN MINERAL	MINERAL PER JAR	RATIO Na:K IN JAR	YIELD OF OATS	UTILIZATION OF ELEMENT ADDED	
						K	Na
	per cent	per cent	gm.		gm.	per cent	per cent
No K					50.6		
Microcline	9.47	1.89	14.13	1:5	52.2	1.12	0.13
Orthoclase	5.72	2.04	23.40	1:3	53.1	2.23	
Oligoclase	0.58	5.47	230.30	10:1	58.7	3.50	
Labradorite	0.38	3.49	350.40	10:1	60.3	3.45	
Albite	0.11	6.19	620.00*	60:1	61.2	7.07	0.46
K_2SO_4					94.5	70.81	

* One-half as much K as in other applications.

A difficulty in the practical use of the low-potassium feldspars is the obvious impossibility of using the large applications necessary to secure the requisite amount of potassium.

Chirikov (12) found in culture experiments that nepheline, biotite and muscovite were good sources of potassium for plants, but not orthoclase. The ineffectiveness of ammonium salts was also shown.

Miller and Van Natta (47) carried out culture experiments in a mixture of river sand and silt loam in the proportion of 8 : 1. Amounts of feldspar were used ranging from 5700 to 23,000 pounds per acre, and compared to 400 pounds per acre of potassium chloride and sulfate. The cultures were grown in 3-gallon jars, and the yields obtained were 10.2 to 12.6 gm. of barley per jar with the feldspar, 11.7 gm. with KCl and 15.7 gm. with K$_2$SO$_4$. These results are quite favorable to the feldspar. Larger applications prevented germination of the seeds.

Brooks and Gaskill (9) report some results of individual seasons' crops in which feldspar compared favorably with kainit in long continued field plat experiments at the Massachusetts Experiment Station, but state that as an average of 21 years' continuous use of feldspar, no decided increases have been obtained. The soil on which these investigations were conducted is probably well supplied with total potassium.

In addition to plant culture experiments, many investigations have been carried out to study the effects of various chemical compounds upon the solubility of the potassium of silicate minerals, some of which will be discussed later.

III. SOME CONSIDERATIONS OF THE NATURE AND SIGNIFICANCE OF THE SILICATE MINERALS

Formation, composition and structure

The silicate minerals are complex compounds, usually considered as salts of the polybasic silicic acids. The more common bases involved are sodium, potassium, calcium, magnesium, aluminum and iron. They are as a class highly insoluble in water. Considered from the standpoint of the molecular proportions of bases and silica, they range from strongly basic to strongly acidic compounds. The proportion of base to acid in the various crystallized minerals depends, of course, upon the proportions in the molten mass in which the crystallization took place.

Primary minerals are to be found quite abundantly in metamorphosed and sedimentary rocks as well as in igneous rocks. Here crystallization has occurred, not in a molten mass, but probably in most cases under the influence of superheated steam at high pressure. The researches of Friedel and Sarasin (25), Daubreé (17), Kuhlmann (41) and others show that many silicate and other minerals may be formed artificially by crystallization in the presence of steam and other gases under pressure. The crystallization occurs at a temperature far below the melting point of the minerals after they are once formed.

The close chemical relationship between carbon and silicon early led to the assumption that the silicates must be similar in structure to organic carbon compounds. The classical investigations of Lemberg (43, 43a), however, indicate quite clearly that no such analogy exists. It is a noteworthy fact that certain bases are usually associated in the silicate minerals, while certain others are seldom or never found together. Thus sodium-calcium feldspars are quite common, as are also potassium-magnesium silicates, while on the other hand calcium and potassium are seldom found together, and sodium-magnesium minerals are practically unknown. Lemberg found in studying the alterations of these minerals that there is a decided antipathy between certain bases, as a result of which it is extremely difficult to induce a combination of such bases as sodium and mâgnesium, for example. Potassium and sodium combine indifferently in the silicate molecule, but to such an extent that sodium-potassium silicates are of fairly common occurrence. These may be merely mixtures of complex sodium silicates and potassium silicates in many cases.

The determination of the structure of silicates is very difficult if not impossible, because they do not possess those properties by the use of which molecular weights are ascertained. The structure is therefore not definitely known in many cases. Cameron and Bell (10) suggest four viewpoints from which these minerals may be considered.

1. They may be considered as combinations of the oxides. Orthoclase, for instance would be $K_2O.Al_2O_3.6SiO_2$. This method of writing the formula, however, gives no clue to the structure of the molecule.

2. They may be molecular compounds, as the alums. But this will not hold for the solution phase; in the solution are found only the simple salts of the various bases.

3. One may consider them double salts of a complex acid, the Si atoms being united through oxygen. Thus orthoclase is a double potassium-aluminum salt of the acid, $H_4Si_2O_8$, or $K_2Al_2(Si_2O_8)_2$,
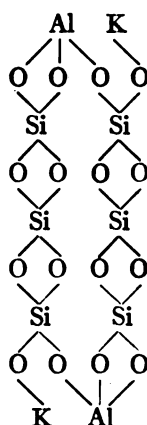
4. Aluminum may be negative, orthoclase being the potassium salt of $H_4AlSi_3O_9$ or $K_2(AlSi_3O_9)_2$,
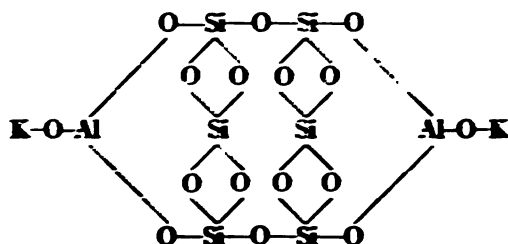
These last two views seem the most reasonable, and the last is preferable in view of the amphoteric nature of aluminum and the formation of the alkali hydroxide as the principal soluble hydrolysis product of the alkali aluminosilicates.

Chemical properties

Reaction with water. The solubility of the complex silicates in water is very low. Stoddart (60) states, for instance, that orthoclase is soluble in 37,000 parts of water, and in 4,000 parts of carbonated water. As early as 1848 W. B. and R. E. Rogers (54) studied the solubility of fifty-eight different minerals in water and in carbonated water. Among these were sodium, lithium and potassium feldspars, micas and leucite. In most cases the solubility was great enough to give good qualitative tests for the bases, and the solubility was greater, as a rule, in water containing CO_2 than in pure water. Simple solution of these minerals in water has never been obtained. Being salts of a very weak acid and very strong bases, solution is always accompanied by hydrolytic decomposition. This reaction is usually arrested almost immediately by the gelatinous coating of colloidal silicic acid on the mineral particles, which prevents further action of the solvent. Clarke (13) found that water reacts immediately with rock powders, producing in most cases a reaction alkaline to phenolphthalein, and Cushman (14) states that equilibrium between the rock powder and water is reached in a very few minutes on shaking the two together. This is obviously not a true chemical equilibrium between solid and solvent, but merely the cessation of reaction due to the interference of the colloidal material on the particles. Daubrée in 1867 (18) overcame the interference of the colloidal material by revolving an iron cylinder containing water and pieces of feldspar and quartz. The sliding motion of the fragments kept fresh surfaces exposed so that the decomposing action of the water was progressive. The solution became alkaline and a fine mud (kaolin) was formed as well as considerable soluble and colloidal material and $FeCO_3$. In 8 days he obtained 12.6 gm. of soluble K_2O from 3000 gm. of feldspar in 5 liters of water. The soluble K_2O he reported present mainly as K_2SiO_3. More recently (1905) Cushman (14) obtained similar results by grinding together

powdered feldspar (also powdered glass) and water made up to a thick paste, in a heavy ball mill. By subsequent washing on a filter paper with water, much more sodium and potassium were dissolved out than where the mineral had been ground dry and shaken with water. He assumed that these bases were present as the simple silicates, since the solution obtained was of a glue-like character. In view of the work of Kahlenberg and Lincoln (38) it is not likely that the silicates of potassium or sodium were present to any marked extent as such, prior to evaporation. These investigators, by studying the freezing point and electrical conductivity of solutions of the simple alkali silicates, showed that the hydrolysis into the hydroxide and colloidal silicic acid is practically complete in concentrations up to 1 gram-molecule of the silicate per 48 liters of solution.

Reaction with carbonic acid. Silicate decomposition by carbonated water results in the formation, in the main, of the same final products as by reaction with pure water, except that the alkalies and alkaline earth metals appear as the carbonates or bicarbonates. Beginning with the early work of Rogers (54) the majority of investigations indicate a greater degree of solubility of the silicate minerals in water containing CO_2 than in pure water. With carbonated water, as with pure water, the amount of direct contact of mineral with reagent is of great importance, as is shown by Bassalik's (4, 5) work. Bassalik studied the solvent action upon various minerals of bacterial activity. Pure cultures of many organisms were grown in suspensions of the minerals, after which the filtrates were analyzed. *Bacillus extorquens*, a CO_2 producer, dissolved from 4 to 40 times as much orthoclase as any other CO_2 producer studied, although some of the others, including yeast, produced much larger amounts of CO_2. This is accounted for by the fact that *B. extorquens* grows directly upon the mineral particles, enclosing the mineral and colony in a zoogleal excresence, thus affording more intimate contact of the CO_2 and the mineral particles. Van Hise (66), in his comprehensive work upon metamorphism, ascribes by far the largest number of natural alterations of rocks to the action of CO_2 and water combined.

Reaction with salts. This reaction might be expected to effect some solution of the potassium of potassium-containing silicates, through the replacement of the mineral potassium by the basic ion of the soluble salt. Lemberg's (43, 43a) careful researches show that reactions of this character take place most rapidly and go much nearer to completion under fusion conditions. When aqueous solutions and suspensions are used, increasing the temperature accelerates the reactions greatly. Exchange of bases was brought about to a considerable degree in most cases within one month at 100°C., but at ordinary temperature the reactions were very incomplete. It may be noted that fusion of silicates with other compounds and also heating such mixtures to varying temperatures with superheated steam under pressure are the bases of many of the patented processes mentioned in another part of this paper. Lemberg also noted that the common bases have different replacing powers, potassium being strongest,

followed by magnesium, sodium and calcium in the order named. Thus it was found much easier to convert a sodium mineral into the corresponding-potassium mineral, than to bring about the reverse reaction. This replacing power holds for the soil mass as well as for the individual minerals, and the point is illustrated by drainage water analyses at Rothamsted (42) and elsewhere, where calcium is present in largest amounts and potassium in the smallest quantities.

All of these observations point to the great difficulty of liberating appreciable quantities of potassium from the primary minerals by means of the so-called exchange of bases, under soil conditions. The liberation in the soil of potassium for plant growth from the primary minerals must be left to the natural process of weathering (19, 31, 61) some of the most important forces of which are the solvent action of water, of CO_2 and of other products of biological activity. Of the last-named, the nitrous acid formed in nitrification is important (4, 5).

Secondary minerals and absorption. When the primary minerals are attacked by various solvent agents the amount of mineral decomposed is greater than would be indicated by the potassium which may be extracted by water (4, 5). This is accounted for as follows. A part of the potassium which is liberated as the hydroxide or carbonate is at once absorbed by the kaolinite formed in the initial reaction (16). This absorption is partly physical, a result of the colloidal nature of the siliceous products of decomposition, but secondary chemical reactions also occur. Similar "absorption-reactions" occur when stable salts of the alkali metals, as the sulfate or chloride, are added to colloidal material. In such cases the metal is usually absorbed as the hydroxide, leaving a pronounced acidity in the solution. While this is the general rule, exceptions occur, depending upon the nature of the colloid and of the salt used (27, 44, 65, 68).

The abundance of colloidal substances and of kaolinite in the average soils makes possible their very great absorptive capacity for potassium. Way (67) was the first to observe the absorption of bases by clay and the practical agricultural application of this phenomenon. Many methods have been devised for determining the so-called "immediately available," "remotely available or reserve" and "unavailable" potassium of soils. These consist for the most part of extraction with water or other solvents such as certain organic and mineral acids of varying concentrations and solutions of various salts. While these methods are purely arbitrary, and for that reason can scarcely be said even to give approximately the availability of soil potassium to the various crops, they do enable one to distinguish roughly the proportions of absorbed potassium, of the potassium of secondary minerals and of that contained in primary minerals in the soil. They are therefore not without value. The work of Fraps and his associates (22, 23) and of Frear and Erb (24) is of interest from this point of view.

The results of chemical researches and of plant culture experiments lead to the conclusions: (a) that the weaker solvents extract the potassium which is contained in the secondary soil minerals and that which may be temporarily held by physical absorption, but only a very small fraction of the potassium of the native minerals; and (b) that while crops may draw upon the unweathered, primary minerals for a portion of their potassium in emergencies, they rely in the main upon the absorbed potassium and secondary minerals for their supply (6, 7, 22, 23, 24, 37, 40, 49, 51, 53, 62, 63).

IV. EXPERIMENTAL

General plan of experiments

The four experiments here reported were carried out for the purpose of obtaining information on the following questions:

1. Will applications of primary potassium-containing minerals to peat soil affect the yield of crops grown therein?

2. To what extent are crops enabled to obtain potassium from those minerals?

3. Is the availability of the mineral potassium for plants affected by the presence of decomposing active organic material, or of magnesium or sodium chloride?

4. What are the effects of decomposing organic material and of magnesium and sodium chlorides upon the amount of potassium which can be extracted by water from these minerals under soil conditions?

5. What effect does decomposing organic matter have upon the solubility of the mineral potassium in the absence of soil?

6. What is the explanation of the low availability of the potassium of dune sand?

Description of materials used

The soil used was a very loose peaty soil obtained from an area several hundred acres in extent near Manito, Illinois. It was quite free from sand and from roots or other residues of recently grown plants. It had been under cultivation for several years but had not been cropped more than once or twice within the last six or seven years. The last fertilizer treatment it had received was an application of KCl 14 years before the collection of soil for these experiments. The reaction was practically neutral, the lime-requirement as determined by the Hopkins method being 285 pounds $CaCO_3$ per acre. It contained 0.411 per cent of total potassium, or 4110 pounds per acre (1 million pounds peat soil assumed as the weight of an acre—6⅔ inches).

The minerals selected for study were orthoclase feldspar from San Diego County, California, microcline from Pennsylvania, leucite rock from the Leucite Hills, Wyoming, alunite from Utah, muscovite from North Carolina and lepidolite from the Black Hills of South Dakota. The impossibility of grind-

ing a large enough amount of muscovite sufficiently fine with the apparatus available necessitated its omission from the experiments. The ignited alunite was prepared by igniting to a bright red heat in a muffle furnace for two hours, until SO_2 fumes were no longer given off. The minerals used contained the following amounts of total potassium (K).

	per cent		per cent
Orthoclase	11.00	Alunite	8.32
Microcline	11.23	Ignited alunite	13.72
Leucite	9.49	Lepidolite	8.99

The limestone used was high-calcium stone containing 0.21 per cent K and approximately 92 per cent $CACO_3$.

The organic materials used were: (a) bright clean alfalfa hay, finely ground (legume), (b) clean prairie hay, consisting mainly of blades, finely ground (non-legume) and (c) fresh cow manure, without litter, carefully dried at low temperature and ground. For experiments II and III the manure was used fresh, without drying.

The chemicals were high-grade analyzed chemicals from the laboratory stock.

Experiment I. Effect of potassium-containing minerals on yield and composition of buckwheat crop

Six series of plant cultures were grown in 4-gallon glazed earthenware jars provided with drainage outlets. Each jar was filled with peat soil equivalent to 3039 gm. water-free soil. All received limestone, finely ground, at the rate of 1 ton per acre, or 12.5 gm. per jar, based upon the area of the jar. Series 100 was a check series and received no minerals. Series 200 to 600 received orthoclase, microcline, leucite, alunite and lepidolite, respectively, at the rate of 25 gm. per jar, or 2 tons per acre. The only exception to this is that jars 1a and 2a in each series received 125 gm., or 10 tons per acre. Additional treatments were applied to the jars of each of the six series as indicated in the following table:

Table showing applications to crop culture jars in addition to minerals

NUMBER OF JAR	SUBSTANCE ADDED	POUNDS PER ACRE	GRAMS PER JAR
1, 2	Nothing		
1a, 2a	Mineral alone, 10 tons	20,000	125.0
1b, 2b	Mineral alone, ignited	2,000	25.0*
3, 4	Alfalfa	6,000	37.5
5, 6	Prarie hay	6,000	37.5
7, 8	Cow manure	3,536	22.1†
9, 10	$MgCl_2.6H_2O$	800	5.0
11, 12	NaCl	500	3.1
13, 14	KCl	200	1.25‡

* Series 500 (alunite) only.

† 22.1 gm. air-dry manure equivalent to 125 gm. fresh manure, or 20,000 pounds per acre.

‡ Series 100 (check) only.

All of the 43 treatments were carried out in duplicate. The jars were planted to buckwheat April 22, 1918, and as soon as the plants were two to three inches high they were thinned to 11 plants per jar. The moisture content was maintained at approximately 160 per cent of the water-free weight of soil. The plants grew vigorously throughout the season, except in series 600, in which they did not thrive as well as in series 100, the next poorest. Numbers 601a and 602a, which received the heavy application of lepidolite were especially stunted, the leaves being small, yellow and curled. Little difference could be observed in the growth of the plants in series 200, 300, 400 and 500.

The crops were harvested on July 25 and preserved in cheesecloth bags until air-dry. They were then weighed, the crops from duplicate jars combined, finely ground, and preserved in tightly stoppered jars for analysis. Table 1 gives the yields from all the jars, the per cent of potassium in the crops, and the amount of potassium removed from each jar.

In experiments II and III the amount of water-soluble potassium which could be extracted from the peat soil by shaking with an excess of water was determined at two different times on aliquots of the same sample, kept under optimum moisture conditions. The water-soluble potassium of the organic materials was determined by the same method. These amounts were:

	Part of total K *which is soluble per cent*
For peat soil, initial, 37.7 mgm. K per 100 gm. water-free soil	9.17
For peat soil 80 days later, 33.9 mgm. K per 100 gm. water-free soil	8.25
For alfalfa, 15.4 mgm. K per gram of dry matter	96.25
For prairie hay, 8.4 mgm. K per gram of dry matter	97.68
For cow manure, 6.6 mgm. K per gram of dry matter	100.00

There are three sources of soluble potassium for the crop, which can thus be measured, that in the soil, that in the organic materials added (or KCl in 113 and 114), and that in the seeds planted. The total potassium added per jar in the seeds planted was 2 mgm., all of which is reckoned as available. By difference the potassium obtained by the crop from insoluble sources can be calculated. These amounts are recorded in the last column of table 1 (+) as well as any excess of soluble potassium in the soil which the crop did not take up (−).

Discussion of results

The relative increases in yield produced by applications of the minerals are quite large, as shown by the percentage calculations recorded in table 2. Lepidolite gives unsatisfactory returns, but with the other four minerals only one case is found in which the yield is less than with the same treatment minus the mineral (jars 209, 210), and here the variation in duplicates is wider than that in the two treatments. That these increases in yield were produced by the mineral rather than by the auxiliary treatment is indicated by table 3.

TABLE 1

Yield and potassium content of buckwheat in experiment I

NUMBER OF JAR	YIELD OF CROP	AVERAGE YIELD	K IN CROP	K REMOVED FROM JAR	SOLUBLE K FROM ALL SOURCES	K OBTAINED FROM INSOLUBLE SOURCES
	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*
		Series 100.	Check			
101	52.9					
102	55.3	54.1	1.96	1.060	1.148	−0.088
103	66.5					
104	55.4	60.9	2.07	1.261	1.725	−0.464
105	46.7					
106	40.2	43.4	2.37	1.028	1.463	−0.435
107	44.5					
108	57.8	51.1	2.24	1.146	1.294	−0.148
109	48.8					
110	54.9	51.8	2.21	1.145	1.148	−0.003
111	37.2					
112	50.8	44.0	2.43	1.069	1.148	−0.079
113	59.3					
114	61.9	60.6	1.88	1.140	1.803	−0.663
		Series 200.	Orthoclase			
201	68.3					
202	76.1	72.2	1.87	1.350	1.148	+0.202
201a	72.1					
202a	66.4	69.2	1.95	1.350	1.148	+0.202
203	65.5					
204	61.8	63.6	2.67	1.698	1.725	−0.027
205	59.7					
206	57.4	58.5	2.23	1.305	1.463	−0.158
207	69.5					
208	71.6	70.5	2.17	1.530	1.294	+0.236
209	42.3					
210	55.1	48.7	2.32	1.130	1.148	−0.018
211	52.6					
212	59.9	56.2	2.34	1.315	1.148	+0.167

TABLE 1—*Continued*

NUMBER OF JAR	YIELD OF CROP	AVERAGE YIELD	K IN CROP	K REMOVED FROM JAR	SOLUBLE K FROM ALL SOURCES	K OBTAINED FROM INSOLUBLE SOURCES

Series 300. Microcline

	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*
301	76.0					
302	75.6	75.8	1.69	1.281	1.148	+0.133
301a	75.5					
302a	65.0	70.2	1.80	1.264	1.148	+0.116
303	75.2					
304	61.9	68.5	1.90	1.302	1.725	−0.423
305	61.7					
306	57.8	59.7	1.95	1.164	1.463	−0.299
307	77.2					
308	68.0	72.6	2.00	1.452	1.294	+0.158
309	60.5					
310	63.0	61.7	1.78	1.098	1.148	−0.050
311	50.6					
312	53.0*	51.8	1.59	0.855	1.148	−0.293

Series 400. Leucite

401	71.2					
402	80.3	75.7	1.63	1.234	1.148	+0.086
401a	79.4					
402a	76.4	77.9	1.75	1.363	1.148	+0.215
403	71.2					
404	69.2	70.2	2.32	1.629	1.725	−0.096
405	78.3					
406	56.1	67.2	1.72	1.155	1.463	−0.308
407	74.0					
408	71.8	72.9	1.66	1.210	1.294	−0.084
409	64.9					
410	66.2	65.5	1.86	1.218	1.148	+0.070
411	59.3					
412	61.6	60.4	2.15	1.298	1.148	+0.150

* Stand of only 2 plants.

TABLE 1—*Concluded*

NUMBER OF JAR	YIELD OF CROP	AVERAGE YIELD	K IN CROP	K REMOVED FROM JAR	SOLUBLE K FROM ALL SOURCES	K OBTAINED FROM INSOLUBLE SOURCES
	gm.	*gm.*	*per cent*	*gm.*	*gm.*	*gm.*

Series 500. Alunite

NUMBER OF JAR	YIELD OF CROP	AVERAGE YIELD	K IN CROP	K REMOVED FROM JAR	SOLUBLE K FROM ALL SOURCES	K OBTAINED FROM INSOLUBLE SOURCES
501	74.7					
502	77.9	76.3	1.73	1.320	1.148	+0.172
501a	72.3					
502a	72.4	72.3	1.72	1.244	1.148	+0.096
501b	63.8					{(−2.999*)}
502b	62.4	63.1	2.47	1.559	1.148	{+0.411 }
503	72.4					
504	66.9	69.6	2.27	1.580	1.725	−0.145
505	62.0					
506	60.1	61.0	1.75	1.068	1.463	−0.395
507	68.7					
508	69.3	69.0	1.92	1.324	1.294	+0.030
509	63.6					
510	72.8	68.2	1.93	1.316	1.148	+0.168
511	67.2					
512	67.0	67.1	1.92	1.288	1.148	+0.140

Series 600. Lepidolite

NUMBER OF JAR	YIELD OF CROP	AVERAGE YIELD	K IN CROP	K REMOVED FROM JAR	SOLUBLE K FROM ALL SOURCES	K OBTAINED FROM INSOLUBLE SOURCES
601	58.7					
602	60.5	59.6	2.06	1.227	1.148	+0.079
601a	27.9					
602a	33.6	30.7	2.39	0.735	1.148	−0.413
603	60.0					
604	50.4	55.2	2.21	1.220	1.725	−0.505
605	52.6					
606	47.6	50.1	2.60	1.302	1.463	−0.161
607	65.2					
608	63.6	64.4	2.00	1.288	1.294	−0.006
609	45.8					
610	47.5	46.6	1.94	0.903	1.148	−0.245
611	51.9					
612	51.8	51.8	1.95	1.010	1.148	−0.138

* This value is obtained if the potassium of ignited alunite is considered entirely available.

In this table the relative yields in each series are calculated as percentages of the yield produced by the mineral alone, which is taken as 100. Almost without exception the yield is greater with the mineral alone than where any of the additional treatments were applied.

The value of the decaying organic manures in liberating potassium is unfairly minimized, however, if yields alone are taken as a criterion. The value

TABLE 2

Relative yields of buckwheat. Effect of minerals (no mineral = 100)

JAR NUMBER	AUXILIARY TREATMENT	SERIES 100, NO MINERAL	SERIES 200, ORTHO-CLASE	SERIES 300, MICRO-CLINE	SERIES 400, LEUCITE	SERIES 500, ALUNITE	SERIES 600, LEPID-OLITE
1, 2	None	100	133.5	140.2	139.9	141.0	110.2
1a, 2a	10 tons mineral		127.9	129.7	144.0	133.7	56.7
1b, 2b	Ignition of mineral					116.6	
3, 4	Alfalfa	100	104.4	112.5	115.3	114.3	90.6
5, 6	Prairie hay	100	134.8	137.6	154.8	140.3	115.4
7, 8	Cow manure	100	138.0	142.1	142.7	135.0	126.0
9, 10	MgCl₂.6H₂O	100	94.0	119.1	126.5	131.7	90.0
11, 12	NaCl	100	127.7	122.3	137.3	152.5	117.7
13, 14	KCl	112					
Average.....		100	121.1	128.3	134.8	133.1	107.3

TABLE 3

Relative yields of buckwheat. Effect of auxiliary treatment (mineral alone = 100)

JAR NUMBER	AUXILIARY TREATMENT	SERIES 100, NO MINERAL	SERIES 200, ORTHO-CLASE	SERIES 300, MICRO-CLINE	SERIES 400, LEUCITE	SERIES 500, ALUNITE	SERIES 600, LEPID-OLITE	AVERAGE
1, 2	None	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1a, 2a	10 tons mineral		95.8	92.6	102.9	94.7	51.5	87.5
1b, 2b	Ignition of mineral					82.7		
3, 4	Alfalfa	112.6	88.1	90.4	92.7	91.2	92.6	94.9
5, 6	Prairie hay	80.2	81.0	78.7	88.8	79.9	84.1	82.1
7, 8	Cow manure	94.4	97.7	95.8	96.4	90.4	108.1	97.1
9, 10	MgCl₂6H₂O	95.7	67.4	81.4	86.5	89.4	78.2	83.1
11, 12	NaCl	81.3	77.8	68.3	79.8	87.9	86.9	80.3
13, 14	KCl	112.0						

of decomposing organic matter in dissolving relatively insoluble mineral plant nutrients is generally ascribed largely to the HNO_3 produced in nitrification, organic acids produced by partial decomposition of non-nitrogenous compounds, and CO_2. The large excess of limestone applied to all the jars furnished an abundance of readily available base for the neutralization of these acids, thus preventing them from attacking the less soluble potassium silicates. This fact was realized at the beginning of the work, but it was believed that

the beneficial effect of the limestone in the soil to the growing crop would cause sufficiently increased root growth and feeding power of the plant to offset the disadvantage. The data of these experiments furnishes no basis for a comparison of these values. Furthermore, the large amount of organic matter of the peat itself was probably decomposed rapidly enough to affect the minerals to almost if not quite as great an extent as the small amount of organic material added. Indications of considerable nitrification were furnished by the appearance occasionally during the growing season, of a slight white or brownish crust of nitrates and other salts on the surface of the soil in all the jars.

An inventory of the sources of potassium, soluble and insoluble, upon which the crops could draw for their supply indicates that the deficiency of available potassium in this soil is not as great as is to be desired in an experiment of this kind. The soluble potassium in the soil alone, according to the extraction and analysis noted previously, amounts to 1.146 gm. per jar. The amount of soluble potassium added in the organic materials is, for the jars receiving those materials, 0.755 gm. in the alfalfa, 0.315 gm. in the prairie hay and 0.146 gm. in the manure. The 0.002 gm. of potassium added in the seed is considered available, and the sum of these three amounts gives the values recorded in the next to last column of table 1. This leaves insoluble potassium in the soil of each jar, 11.314 gm.; in the limestone added 0.026 gm. and in the minerals added, 2.08 to 2.81 gm. except where the 10-ton applications were made, in which there was five times as much. In calculating the values for the last column of table 1 it was assumed that the crop utilizes all the potassium from the soluble sources named before beginning on the insoluble supply. It may be noted that in 25 of the 43 cultures, a part of the soluble potassium amounting to from 3 to 663 mgm. remained in the jars, unused by the plant, while in only 18 jars the plants utilized a part of the insoluble potassium. These unused reserves of soluble potassium were present mainly in series 100 and 600, where the yields were small, and the crops more or less unthrifty in appearance. In the other four series the unused reserves were found, mainly, in the jars receiving an excess of soluble potassium in the organic manures added.

The percentage of potassium in the crops from the various treatments varies widely. These variations appear to depend as much upon the available supply of potassium as upon the crop yield. The total amount removed per jar, on the other hand, is necessarily dependent upon the yield to a large extent. As a result, the high yields are accompanied by the removal of some potassium from the sources listed as insoluble, except where an abundant supply of soluble potassium has been added in the organic materials. The maximum amount of "insoluble" potassium thus taken up by the plants, was in jars 401a and 402a, where the potassium from insoluble sources constituted 15.8 per cent of the total amount taken up by the crop.

The effect of the minerals upon the amount of potassium utilized by the crop may be shown very clearly by averaging the amounts of potassium removed per jar for each series separately. The following table gives these averages,

as well as the relative amounts removed per jar on a basis of 100 for the series receiving no mineral:

SERIES	K REMOVED PER JAR, AVERAGE OF 14 JARS	RELATIVE AMOUNT OF K REMOVED PER JAR, AVERAGE OF 14 JARS
	gm.	
100, No mineral	1.118	100.0
200, Orthoclase	1.383	123.7
300, Microcline	1.202	107.5
400, Leucite	1.301	116.3
500, Alunite	1.306*	116.8*
501b, 502b, Ignited alunite	1.559	139.5
600, Lepidolite	1.098	98.26

* Average of 16 jars.

Increased amounts of potassium are thus seen to be removed by the crops, amounting to 7.5 to 23.7 per cent where minerals are supplied, if we exclude ignited alunite, which contains much soluble potassium, and lepidolite, which shows unsatisfactory results here as in the yields. This method of averaging is not unfair, because the values averaged are from jars receiving the same supplementary treatments in each series.

These observations lead to the following conclusions:

1. Applications of 25 gm. per jar (approximately 2 tons per acre) of orthoclase, microcline, leucite and alunite increase the yield of buckwheat from 20 to 35 per cent and also enable the crop to utilize all the water-soluble potassium present as well as some of the less available forms. A part of this may come from the minerals applied.

2. On the peat soils used in this experiment the addition of crop residues, manure, or soluble magnesium or sodium salts does not increase the yield of buckwheat or the availablility of the potassium of the minerals added.

3. The activities of the root system through contact with the soil or mineral particles, and commonly spoken of as the "feeding power" of plants, is an important factor to be considered in the liberation of relatively insoluble potassium.

Experiment II. Solubility of the minerals in water as affected by decaying organic materials and soluble salts under soil conditions

This experiment was carried out in half-gallon Mason jars, loosely covered in order to prevent excessive evaporation and keep out dirt, without excluding air. It was the purpose to follow the general plan of Experiment I as to treatments, but to determine the potassium in a water extract instead of growing a crop in the soil. Since the absorbing power of a soil prevents as complete extraction of the water-soluble potassium as the plant is capable of, a larger proportion of mineral to soil was used than in the culture experiment. The

method of shaking the soil with a large excess of water was chosen in preference to the percolation method of extraction in order to lessen potassium absorption as much as possible.

To each 100 gm. of water-free soil was added enough mineral to carry 1 gm. of potassium. The organic materials were added at the rate of 1 gm. of dry matter per 100 gm. of soil and the soluble salts were added in molecular equivalents of the potassium contained in the mineral. In addition to the auxiliary treatments used in experiment I, NH₄Cl and dextrose were used. Table 4 gives the treatments in detail.

In each case double the amount of soil needed for an extraction was taken. The minerals and other substances were added to the air-dry soil (except soluble salts and dextrose) and well mixed, dry. Enough distilled water was

TABLE 4

Soil and treatments used in experiment II

JAR NUMBER	PEAT SOIL EQUIVALENT TO WATER-FREE SOIL	AUXILIARY TREATMENT		SERIES A, NO MINERAL	SERIES B, ORTHO-CLASE	SERIES C, MICRO-CLINE	SERIES D, LEUCITE	SERIES E, ALUNITE	SERIES F, LEPID-OLITE
		Substance added	Amount						
	gm.		*gm.*		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	200	Nothing			18.32	17.81	21.07	24.04	22.24
2	200	CaCO₃	2.56		18.32	17.81	21.07	24.04	22.24
3	400	Alfalfa	4.26*		36.64	35.62	42.14	48.08	44.48
4	400	Prairie hay	4.29*		36.64	35.62	42.14	48.08	44.48
5	400	Fresh manure	23.91*		36.64	35.62	42.14	48.08	44.48
6	200	MgCl₄.6H₂O	5.20		18.32	17.81	21.07	24.04	22.24
7	200	NaCl	2.99		18.32	17.81	21.07	24.04	22.24
8	200	NH₄Cl	2.74		18.32	17.81	21.07	24.04	22.24
9	200	Dextrose	2.00		18.32	17.81	21.07	24.04	22.24
10	200	KCl	3.81						

* Equivalent to 4.00 gm. dry matter.

added to bring up to optimum (160 per cent) and again very thoroughly mixed. The soluble salts and dextrose were dissolved in the water used for making the soil up to optimum. The entire mass was then weighed, one-half removed for extraction and the other half placed in the Mason jars. The fractions kept in the jars were weighed and brought up to optimum moisture content every few days with distilled water during the period of the experiment. The double amounts of soil prepared in jars 3, 4 and 5 of each series were prepared in order that ammonia and nitrate nitrogen might be determined in the soils receiving organic manures. This might throw some light upon a possible correlation of ammonification, nitrification and solution of the potassium of the minerals used. Unfortunately, the determinations were all ruined by the breaking of a refrigerating machine and consequent flooding of the building with NH₃ fumes. A few nitrate determinations were saved but the results were not considered of sufficient importance to record.

The method of determining water-soluble potassium was as follows. The moist treated soils were immediately placed in 2½-liter bottles, shaken 5 hours in a mechanical shaker with 1500 cc. distilled water (including the water already contained in the sample) and then allowed to settle 36 hours. The supernatant solution was then filtered through filter paper and placed in glass stoppered bottles with a few drops of chloroform until used. Aliquots of this solution were clarified by shaking with 5 cc. of milk of lime. Aliquots of the

TABLE 5

Milligrams of potassium extracted by water from 100 grams of soil

JAR NUMBER	TREATMENT	A, CHECK NO MINERAL	B, ORTHO-CLASE	C, MICRO-CLINE	D, LEUCITE	E, ALUNITE	F, LEPID-OLITE
		mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1	Nothing	37.7	26.6	26.6	32.0	28.5	39.8
		33.9	33.5	32.3	37.3	33.7	52.6
2	$CaCO_3$	32.9	33.7	32.0	35.3	32.4	46.2
		29.1	33.1	30.1	36.9	33.5	46.1
3	Alfalfa	39.1	40.3	34.6	39.6	35.7	51.9
		41.0	42.6	40.3	45.3	40.6	62.3
4	Prairie hay	31.7	32.8	28.8	30.7	31.4	50.8
		34.4	36.1	33.5	39.6	36.0	49.5
5	Manure	27.7	31.4	27.6	31.2	27.8	48.8
		33.0	36.4	35.0	39.8	35.3	53.1
8	NH_4Cl	29.8	43.7	41.3	45.9	42.4	62.9
		43.9	44.0	45.2	56.3	46.0	84.4
9	Dextrose	23.6	26.6	26.3	21.2	29.7	49.6
		29.7	31.2	28.1	37.4	31.1	48.4
10	KCl	184.6					
		176.4					

clarified solution were then filtered and taken to dryness in an excess of H_2SO_4, ignited to destroy organic matter, taken up in boiling water and thoroughly triturated with an agate pestle. Calcium sulfate was filtered off and washed, the remainder of the calcium was removed as the oxalate, ammonium salts driven off and potassium determined gravimetrically as K_2PtCl_6.

At the end of 80 days the second set of moist soil samples was removed from the jars, extracted and the extract analyzed for potassium in the same way. It was impossible in the limited time available to overcome difficulties caused by the interference of the large amounts of magnesium and sodium in numbers 6 and 7. The results are considered untrustworthy and are therefore not presented.

The results of the analyses are recorded in table 5, the figures representing milligrams of K per 100 gm. of water-free soil, or parts per 100,000. The upper row of figures represents the results from the initial water-extraction, and the lower row, results of extraction after 80 days of moist contact of soil and mineral under aerobic conditions.

Subtracting the values given in the check column, A, from those in each of the succeeding columns gives the increase in soluble potassium due to the addition of the minerals. These values are in table 6.

TABLE 6

Gains (+) in soluble potassium over check

JAR NUMBER	TREATMENT	MGM. K PER 100 GM. SOIL				
		B, Orthoclase	C, Microcline	D, Leucite	E, Alunite	F, Lepidolite
1	0	−11.1	−11.1	− 5.7	− 9.2	+ 2.1
		+ 0.4	− 1.6	+ 3.4	− 0.2	+18.7
2	$CaCO_3$	+ 0.8	− 0.9	+ 2.4	+ 0.5	+13.3
		+ 4.0	+ 1.0	+ 7.0	+ 4.4	+17.0
3	Alfalfa	+ 1.2	− 4.5	+ 0.5	− 3.4	+12.8
		+ 1.6	− 0.7	+ 4.3	− 0.4	+21.3
4	Prairie hay	+ 1.1	− 2.9	− 1.0	− 0.3	+19.1
		+ 1.7	− 0.9	+ 5.2	+ 1.6	+15.1
5	Manure	+ 3.7	− 0.1	+ 3.5	+ 0.1	+21.1
		+ 3.4	+ 2.0	+ 6.8	+ 2.3	+20.1
8	NH_4Cl	+ 3.9	+11.5	+16.1	+12.6	+33.1
		+ 0.1	+ 1.3	+12.4	+ 2.1	+40.5
9	Dextrose	+ 3.0	+ 2.7	− 2.4	+ 6.1	+26.0
		+ 1.5	− 1.6	+ 7.7	+ 1.4	+18.7

Discussion of results

The most striking fact brought out by table 5 is the large amount of soluble potassium in the untreated peat soil. From 8 to 9 per cent of the total potassium in the soil is extracted by shaking with water. This suggests that a considerable proportion of the soil potassium of the peat is held by the organic matter of the soil, since potassium which is bound to the mineral soil constituents is, as a rule, much more firmly held.

An apparent increase in the amount of soluble potassium after 80 days' standing is noted throughout table 5, almost without exception.

The effects of the minerals are most clearly shown in table 6. Three points are conspicuous.

(a) The solubility of lepidolite is very high throughout all the treatments. The solubility of lepidolite is influenced little or not at all by any of the treatments. In view of this result, the stunting of the crop grown in series 600 of experiment I may well be ascribed to the presence of some toxic element dissolved out of the mineral, probably an excess of soluble lithium. The excess of calcium carbonate in the soil, which was already practically neutral makes poisoning by soluble aluminum salts appear improbable.

(b) The addition of the minerals to the untreated soil (no. 1, table 6) caused an immediate disappearance of considerable amounts of potassium from solution which reappeared in the later extraction. This suggests a possible temporary absorbing capacity of the minerals themselves for potassium.

(c) The only evidence of potassium liberation by exchange of bases is furnished by the extraction with NH_4Cl. With the exception of orthoclase there is an increased amount of potassium dissolved to the extent of 11 to 33 mgm. per 100 gm. of soil over that with NH_4Cl alone (no mineral). The increase over the mineral alone is equally marked, as shown in table 5. This is in accord with the findings of André (2), Steiger (59) and Beyer (6). The solubility in all the other treatments is but slight, the values recorded being, for the most part, within the limits of experimental error. Leucite, however, shows distinctly greater solubility than orthoclase, microcline or alunite.

Concerning the auxiliary treatments, nothing can be said in their favor, used under the conditions of this experiment, except for the NH_4Cl already noted. Where the organic manures have been added, there is an increase in potassium extracted, but it should be noted that the increase is not sufficient to cover the amount added in the organic material. In every case the soil-mineral mixture has absorbed half or more of the soluble potassium added in the organic manures. Dextrose has depressed the solution of potassium throughout. $CaCO_3$ has had no effect except to decrease slightly the temporary absorption of potassium by the soil-mineral mixture. The absorptive power of the soil is shown in no. 10, table 5. The amounts of soluble potassium found indicate the immediate absorption by the soil of 81.5 per cent of the potassium added as KCl, this being increased to 82.4 per cent during the 80 days.

Experiment III. Solubility of minerals in water as affected by certain decaying organic materials

The general plan of experiment III is the same as in experiment II.

In experiment III mixtures of the minerals and the organic materials used in experiment II were allowed to stand moistened, but not saturated with water for 80 days. The method of mixing and of extracting was exactly the same as in experiment II. Each mineral was treated with two different proportions of the organic material. One was the same as in experiment II, namely, 1 gm. dry organic material to 1 gm. total K in mineral, while the other mixture contained 10 times as much organic matter. For the purpose of com-

parison, each of the organic materials and minerals was extracted with water separately. The results of these extractions are given in tables 7, 8, and 9. In table 7 the upper row of figures (a) represents the values obtained at the

TABLE 7

Water-soluble potassium in organic matter-mineral mixture

NUMBER	ORGANIC MATERIALS TAKEN	DRY MATTER		MGM. K PER 1 GM. TOTAL K IN MINERAL a = initial b = 80 days later (Mineral used = 10 gm. Total K)				
				B, Ortho-clase	C, Micro-cline	D, Leucite	E, Alunite	F, Lepid-olite
		gm.						
10	Alfalfa	10	a	17.8	16.5	19.2	16.5	36.0
			b	17.0	16.4	19.0	17.7	19.9
11	Alfalfa	100	a	139.1	132.1	134.0	129.2	150.0
			b	159.6	165.8	159.9	154.7	170.7
12	Prairie hay	10	a	11.0	9.5	12.7	9.3	22.5
			b	9.7	8.6	11.8	9.8	12.9
13	Prairie hay	100	a	72.5	82.1	82.8	85.2	103.7
			b	81.5	80.8	85.4	80.5	77.0
14	Manure	10*	a	8.0	7.6	10.4	6.9	19.3
			b	7.7	8.0	11.0	7.1	13.7
15	Manure	100*	a	49.4	30.7	42.3	63.4	78.3
			b	57.2	58.4	69.0	68.6	69.7

* Equivalent amount of fresh manure was used.

TABLE 8

Water-soluble potassium in organic materials used

SUBSTANCE	TOTAL K	WATER-SOLUBLE K	PER CENT OF TOTAL K WHICH IS SOLUBLE
	*per cent**	*per cent**	
Alfalfa..	1.60	1.54	96.25
Prairie hay....................................	0.86	0.84	97.68
Fresh manure.................................	0.66	0.66	100.00

* Percentage in dry matter.

initial extraction, and the lower row (b) the values obtained 80 days later. In order to show the gain or loss of soluble potassium occasioned by contact of the minerals and organic matter, the sum of the values given in tables 8 and 9 was deducted from those in table 7. Table 10 gives the differences.

Discussion of results

An examination of the data in table 10 reveals the following facts. Only where the larger amounts of organic materials are used is there a significant change in the amount of soluble potassium obtained. This is a disappearance of potassium from solution in all the initial extractions and in the later extrac-

TABLE 9

Water-soluble K in minerals

SUBSTANCE	MGM. SOLUBLE K PER 100 GM. MINERAL	MGM. SOLUBLE K PER 1 GM. TOTAL K
Orthoclase..	11.99	1.1
Microcline..	11.79	1.0
Leucite...	42.73	4.5
Alunite...	11.18	1.3
Lepidolite..	62.99	7.0

TABLE 10

Gain or loss of soluble K occasioned by mixing of organic materials with minerals

a = initial   b = 80 days later

NUMBER			B, ORTHOCLASE	C, MICROCLINE	D, LEUCITE	E, ALUNITE	F, LEPIDOLITE
			mgm.	mgm.	mgm.	mgm.	mgm.
10	{	a	+ 1.3	+ 0.1	− 0.7	− 0.2	−13.6*
		b	+ 0.5	0	− 0.9	+ 1.0	− 2.5*
11	{	a	−16.0*	−13.0*	−24.5*	−26.1*	−11.0*
		b	+ 4.5*	+10.8*	+ 1.4	− 0.6	+ 9.7*
12	{	a	+ 1.5	+ 0.1	− 0.2	− 0.4	+ 7.1*
		b	+ 0.2	− 0.8	− 1.1	+ 0.1	− 2.5*
13	{	a	−12.6*	− 2.9*	− 7.7*	− 0.1	+12.7*
		b	− 3.6*	− 4.2*	− 3.1*	− 4.8*	−14.0*
14	{	a	+ 0.3	0	− 0.7	− 1.0	+ 5.7*
		b	0	+ 0.4	− 0.1	− 0.8	+ 0.1
15	{	a	−17.7*	−36.3*	−28.2*	− 3.9*	+ 5.3*
		b	− 9.9*	− 8.6*	− 1.5	+ 1.3	− 3.3*

* The values so marked are the only ones in which the differences are greater than can be accounted for by experimental error.

tions of prairie hay and manure, in amounts up to 55 per cent (C15, initial) of that added in the organic material. Alfalfa during 80 days effected the solution of 4.5 mgm. K per gm. of total K in orthoclase, 10.8 mgm. in microcline and 9.7 mgm. in lepidolite. The greater solvent action of the alfalfa is due in part to the fact that it was decomposed much more rapidly than the other two

organic materials. Four weeks after the beginning of the experiment the alfalfa had broken down to such an extent that none of the plant structure was distinguishable, and the mass resembled the cow manure in appearance. A large proportion of the prairie hay, on the other hand, was but little changed in appearance even at the end of the 80 days. The high proportion of easily decomposable nitrogenous material in the alfalfa may also have been a factor affecting its solvent action. There is a possibility that this factor was really the presence of ammonia, since conditions were more favorable for ammonification than for nitrification.

The high solubility of lepidolite noted in experiment II is further illustrated here, but the potassium dissolved disappears from solution later, as shown by the second extractions. The slow absorption of dissolved potassium suggests

TABLE 11

Absorption of potassium by minerals. Milligrams of K per gram of total K in mineral

NUMBER	MINERAL	AMOUNT OF MINERAL USED (= 1 gm. K)	SOLUBLE K ADDED AS KCl	SOLUBLE K IN MINERAL (TABLE 9)	TOTAL SOLUBLE K	SOLUBLE K FOUND	GAIN (+) OR LOSS (−) OF SOLUBLE K
		gm.	mgm.	mgm.	mgm.	mgm.	mgm.
1	Orthoclase	9.16	150	1.1	151.1	144.6	− 6.5
2	Orthoclase	9.16	300	1.1	301.1	290.2	−10.9
3	Orthoclase	9.16	5	1.1	6.1	6.4	+ 0.3
4	Microcline	8.906	150	1.0	151.0	139.7	−11.3
5	Leucite	10.54	150	4.5	154.5	140.3	−14.2
6	Alunite	12.02	150	1.3	151.3	143.6	− 7.7
7	Lepidolite	11.12	150	7.0	157.0	137.1	−19.9
8	Lepidolite	11.12	225	7.0	232.0	210.1	−21.9

chemical reaction with the mineral, possibly a replacement of lithium by potassium.

The disappearance of potassium from solution in this experiment can be accounted for in only two ways. Either the potassium of the organic matter, upon decomposition of the latter, is in part converted into insoluble forms, or it is absorbed by the mineral. In the case of the initial extractions, the disappearance of soluble potassium can be accounted for only by absorption by the minerals (see table 8).

To verify this point the following experiment was carried out. Portions of each of the minerals equivalent to 1 gm. of potassium were weighed out, definite amounts of soluble potassium added as KCl from an accurately made standard solution and made up to a volume such that the proportion of mineral to water was the same as in the extractions of experiment III. These were shaken 5 hours and allowed to stand 36 hours. Suitable aliquots of these extractions were then analyzed.

The results of this experiment are presented in table 11.

The results of this experiment furnish ample verification of the statements made above. The minerals have absorbed potassium in every case, except where the very small amount of potassium was added in no. 3. The amounts may fairly be compared with those in the upper row, a, of no. 11, table 10. Absorption by the mineral thus accounts for a large part of the potassium which disappeared from solution—more than half except for orthoclase and alunite.

Experiment IV. Availability of Potassium in Sandy Soils

In another part of this paper certain sandy soils of Illinois containing a large amount of potassium were mentioned as being similar to peat soils in one respect, i.e., in that they are deficient in available potassium. Experiment IV was undertaken in order to determine the reason for the low availability of the potassium in such soils. The cultural treatments consist of (a) whole sand as the cultural medium, (b) coarse separates (sands) and (c) a mixture of the

TABLE 12

Total and water-soluble K in the different separates of sandy soil

SUBSTANCE ANALYZED	TOTAL K	WATER-SOLUBLE K PER 100 GM. SOIL
	per cent[*]	*mgm.*
Whole sand, unground..............................	1.03	3.5
Coarse separates, 100-mesh	1.01	5.9
Fine separates	1.59	39.8

[*] Samples ground to impalpable powder for total potassium determination.

coarse separates and the same finely ground. Each jar receives a complete nutrient solution minus potassium.[1]

The whole sand and coarse and fine separates were examined in the laboratory as follows. A mechanical analysis showed that the sandy soil used contained 96.7 per cent of coarse separates (sands) and 3.3 per cent of fine separates (silt plus clay). Separations were made by sedimentation in distilled water. Determinations were made in the three materials of total potassium and also of water-soluble potassium. The latter was determined as in the preceding experiments by shaking together 100 gm. of soil and 1500 cc. of distilled water, potassium being determined in an aliquot of the extract. The results are given in table 12.

In view of the fact that the coarse separates were obtained by sedimentation in water, no water-soluble determination was made on this fraction, unground. The large volume of water necessary to wash out all the silt and clay from the sand (approximately 100 liters for the 100 gm. of silt and clay used in the extraction above) would wash out all soluble potassium, which would remain in the silt and clay on evaporation.

[1] The crop is not yet harvested.

The high absorbing power of the fine separates is here illustrated. The 3.5 mgm. of potassium soluble in the whole sand, when concentrated in the fine separates is not extracted, the fine separates from 100 gm. of sand yielding to extraction only 1.31 mgm. of potassium. Grinding the coarse separates liberates some potassium, but not enough to be of any practical value. The grinding of 1 ton of coarse separates to 100-mesh size and down would produce, according to the figures in table 12, 0.114 pound of soluble potassium.

The relatively small surface of the coarse particles of sandy soils of the type used in this experiment readily accounts for the low availability of the potassium in these soils (29).

V. SUMMARY

1. The use of finely-ground potassium-bearing minerals increases the yield of buckwheat in peat soil 21 to 34.8 per cent.

2. Lepidolite is detrimental to the growth of buckwheat, especially if present in large amounts.

3. The addition of crop residues, manure, or soluble sodium or magnesium salts to peat soil, together with the minerals used in this experiment, does not increase the yield of crop or the availability of the mineral potassium.

4. The so-called "feeding power" of the plant itself, through the activities of the root system, is an important factor in the utilization of relatively insoluble potassium.

5. The solubility of the minerals used, as determined by extraction with water, is very low, except that of lepidolite. The solubility is increased by ammonium chloride and also by the decomposition of alfalfa. The result in the latter case may be due to ammonification of the nitrogenous compounds in the alfalfa.

6. All the minerals used have the ability to absorb considerable amounts of potassium from solution. The absorption is probably physical to a large extent.

7. The low availability of the potassium of dune sand is due to the fact that most of the potassium is contained in the larger particles. The relatively small surface of the particles is sufficient explanation of the low solubility of the potassium contained in them.

8. The increase in solubility of the potassium in such sands produced by grinding is not sufficient to be of practical value.

9. The results of the crop culture work should not be taken as conclusive. They should be verified by repetition for several seasons with various other farm crops.

ACKNOWLEDGMENT

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RELATION OF THE MOISTURE EQUIVALENT OF SOILS TO THE MOISTURE PROPERTIES UNDER FIELD CONDITIONS OF IRRIGATION

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In studies of the water requirement of soils under irrigation, both for land being irrigated and for land for which the construction of irrigation systems is contemplated, some criterion which will furnish an index of the soil moisture properties is needed. Mechanical analyses of the soil are expensive to make and the results, given in the seven grades of soil particles, do not lend themselves to ready comparison except by general soil classes. A criterion is needed which can be expressed as a single factor and which can be determined fairly readily at small expense. The moisture equivalent as suggested by Briggs and McLane is intended to be such an index of the soil moisture properties.

Comparisons of the moisture equivalent with other soil properties have been made, usually under laboratory conditions. The following discussion is a comparison of the moisture equivalent with the critical moisture points of soils under the actual field conditions of irrigation practice, and is based on data secured by the author in the course of various field investigations of the water requirements of different soils and their irrigation practice. This work was done partly while the writer was in the employ of the Irrigation Investigations of the United States Department of Agriculture, but more largely in connection with his general engineering practice. In all cases, the determinations of the moisture equivalents have been made by the Division of Soil Technology of the University of California under the direction of Prof. C. F. Shaw.

The general field method has been to take soil moisture samples before and after irrigation in order to determine the amount of water retained by the soils. Notes on the soil and crop conditions were secured and special samples at wilting taken when feasible. The main purpose of the field work was the study of the water requirements of the soils under irrigation practice. The comparison discussed here was incidental to this main purpose, so that in many cases samples representing only a part of the moisture properties of a particular soil were secured.

The data given were secured from a wide range of soils under varying conditions of practice. In 1913 and 1914, about 7000 individual moisture deter-

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minations from 44 fields with 9 moisture equivalents of typical soils were secured, near Billings, Montana.   In 1915 about 700 individual soil moisture samples and 14 moisture-equivalent determinations were secured during a study of sandy soils on the Minidoka project in Idaho.   The field work in these two investigations was done by the author for the United States Department of Agriculture.   In 1917 about 1000 moisture and 54 moisture-equivalent determinations were secured in a study of a wide variety of soils on the Sunnyside project in Washington.   In 1917 also about 450 soil-moisture and 50 moisture-equivalent samples were secured from irrigated lands near Reno, Nevada.   In addition less extensive results were secured in 1918 on soils near Los Molinos, California and the results obtained by Israelsen (1) for soils in the Sacramento Valley, California, were used.   The results discussed cover a total of 136 determinations of moisture equivalents varying in numerical value from 4.1 to 37.6.

Comparisons of four moisture conditions are made both for the surface foot of soil and for the average of the upper 5 feet of soil.   These are the maximum field capacity, the normal field capacity, the usual moisture before irrigation and the wilting of the crop.   The maximum field capacity applies to soils shortly after irrigation before the moisture distribution is complete or to soils where downward percolation is retarded by heavier soil strata.   It does not cover soil saturation but represents a higher moisture content than would be secured under normal conditions.   The normal field capacity would apply to soils of uniform character at from 1 to 3 days after irrigation when moisture distribution had become fairly complete, although both evaporation from the soil and deep percolation may be continuing but at a lessened rate.   The usual moisture before irrigation represents the minimum moisture under good practice where irrigation would be given just ahead of the actual needs of the crops.   The wilting of the crop represents actual injury, practically the minimum to which moisture may be reduced.

In assembling the observations the soil moisture results were expressed in terms of the percentage of the moisture equivalent.   The moisture equivalents were grouped by variations of 2.5 per cent.   In the figures given the individual results are plotted and also the mean result for each group, the numbers with the means indicating the number of observations included in the mean.   As all four soil-moisture points were not determined for the larger proportion of the individual soils, the number of points in the means is less than the total number of moisture-equivalent determinations in each group. This is particularly true for the means for the 5-foot depths of soil, as many soils included in the field work were of shallow depth or variable subsoil which prevented securing means for the full 5 feet.

In figure 1 the relation of soil moisture in the surface foot under field conditions to the moisture equivalent is given for the four moisture points.   In order to indicate the variation of the individual results broken lines are drawn which represent 10 per cent variation from the mean curves.   The greater
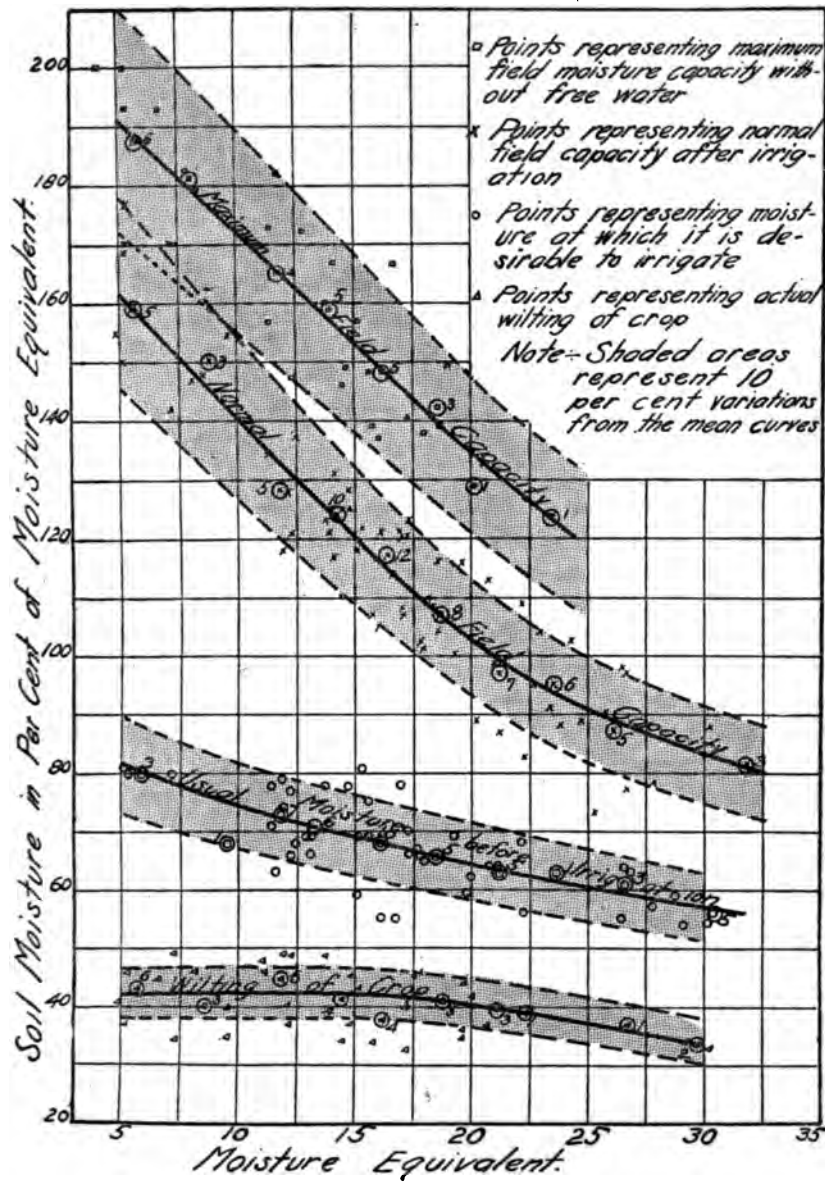
FIG. 1. RELATION OF SOIL MOISTURE IN SURFACE FOOT UNDER FIELD CONDITIONS TO THE
MOISTURE EQUIVALENT

proportion of the observations fall within 10 per cent of the mean. The general relationships appears to be fairly consistent.

The variations of individual results may be due either to a lack of consistency of the moisture equivalent as an index of moisture properties or to a lack of accuracy in the selection of soil moisture samples representing the critical moisture points given. In the author's opinion, the latter source of error is the more probable one. The moisture condition at which a soil needs irrigation is not an exact one, particularly when the surface foot only is considered. It will vary for a given soil with the character of crop and its state of growth and with the moisture and soil conditions in the subsoil. Wilting is also a progressive process and the point at which the crop will fail to revive is difficult of actual determination. After irrigation, soil evaporation will continue at diminishing rates over several days, deep percolation may also continue for a considerable period. During this time the crop is withdrawing moisture for its use so that there is no definite point at which soil moisture samples can be expected to give the exact amount of moisture available for crop use.

It is thought that the results as plotted in figure 1 indicate as consistent a relationship between the moisture equivalent and the soil moisture under field conditions as is to be expected under the circumstances under which the observations were made. The purpose of the comparison was to determine whether such a general relationship exists rather than to express the relationship in definite numerical terms, and any specific numerical deductions from these curves, such as those given later in this discussion, should be considered as suggestive only and as subject to modification as additional numerical data may become available.

In figure 2 curves similar to those in figure 1 are given, except that the comparison is based on the mean moisture in the upper 5 feet of soil. The number of points available was less than of those used in figure 1 and the resulting mean curves are in consequence less definite in both their general form and their actual location than those for figure 1.

The curves given indicate that the relationship between field moisture properties and the moisture equivalent is not a linear one except possibly for conditions approaching wilting. Briggs and McLane have derived the formula

Wilting coefficient = moisture equivalent ÷ 1.84.

This formula was based on experiments where the plants were grown in limited volumes of soil rather than under normal field conditions. The above formula is equivalent to the wilting coefficient equalling 54.4 per cent of the moisture equivalent. The results given on figures 1 and 2 indicate that, at least under field conditions, the crops can reduce the soil moisture of both the surface foot and of the mean for the upper 5 feet below this amount. The mean of all observations on wilting for the surface foot of soil is about 40 per cent of the moisture equivalent.
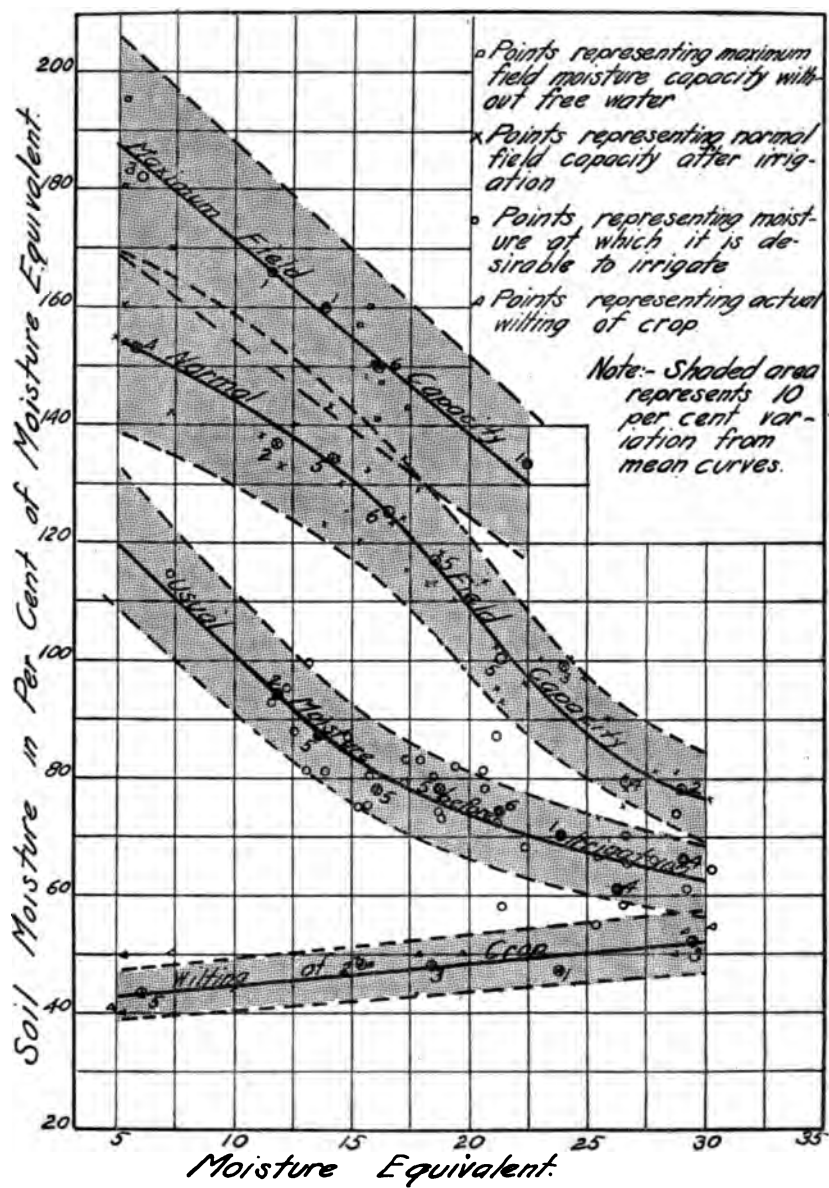
FIG. 2. RELATION OF SOIL MOISTURE IN UPPER 5 FEET OF SOIL UNDER FIELD CONDITIONS
, TO THE MOISTURE EQUIVALENT

Similarly Briggs and McLane have given the equation

$$Moisture\text{-}holding \; capacity = moisture \; equivalent \times 1.57 + 21.$$

The moisture-holding capacity used in this formula is based on the Hilgard short-tube method and exceeds the moisture capacity under field conditions of soils free to drain. In no case does the maximum field capacity reach the amount indicated by this formula.
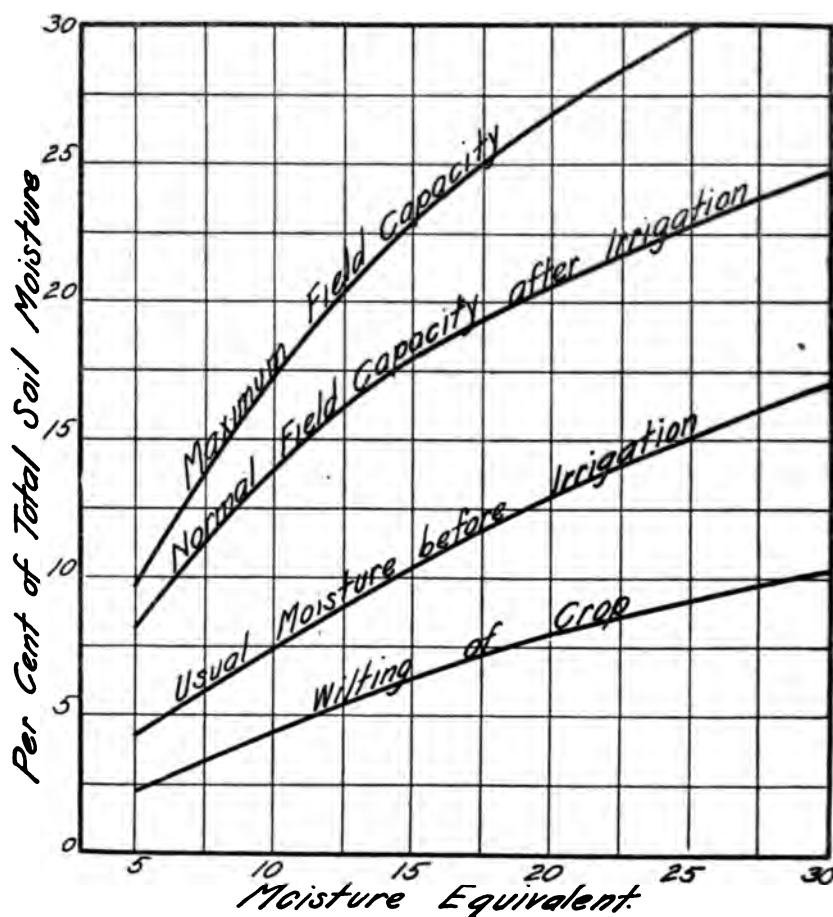
FIG. 3. RELATION OF PER CENT OF TOTAL MOISTURE IN SURFACE FOOT OF SOIL UNDER
FIELD CONDITIONS AND MOISTURE EQUIVALENTS

In figures 3 and 4 the mean curves of figures 1 and 2 are redrawn, the percent of soil moisture being used directly instead of as a percentage of the moisture equivalent. These curves indicate the percentages of soil moisture on the oven-dry basis at the different critical-moisture points.

In figures 5 and 6 the curves of figures 3 and 4 are used to give the water added by usual irrigations.   In figure 5 is plotted the difference in moisture before and after irrigation as shown for usual practice on figures 3 and 4. This shows the largest moisture capacity for 5 feet of soil in soils of medium texture.   The coarse soils having a low moisture equivalent have a relatively small moisture-holding capacity.   The heavy soils while having a
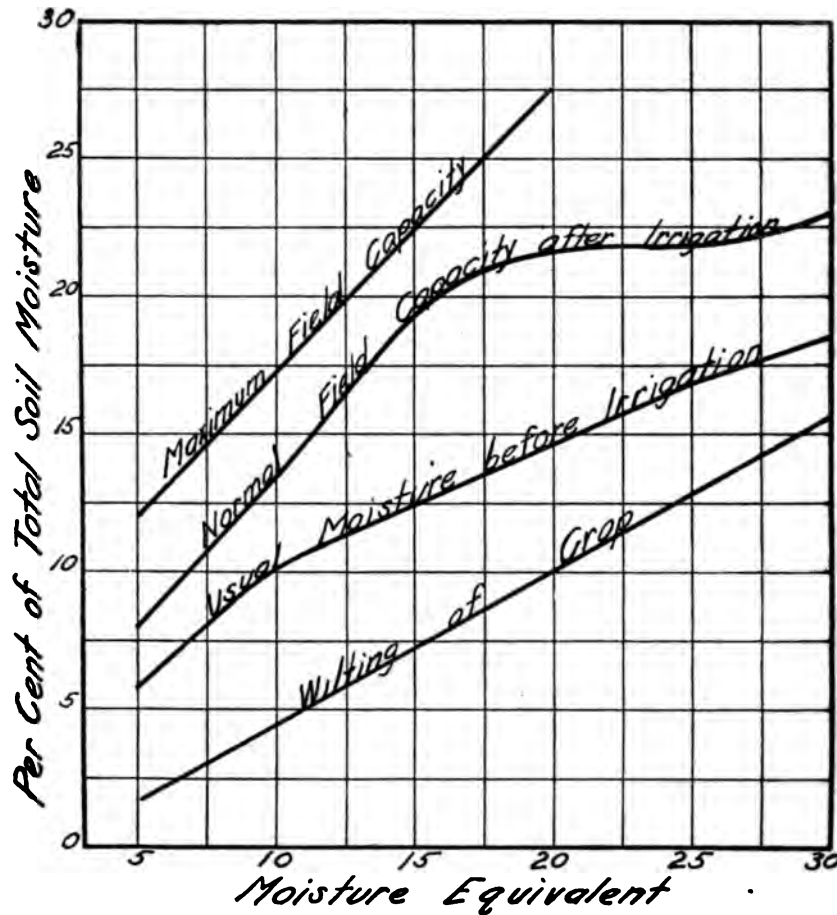
Fig. 4. Relation of Per Cent of Total Moisture in Upper 5 Feet of Soil Under Field Conditions and Moisture Equivalents

relatively large moisture-holding capacity, are not able to utilize this capacity to the 5-foot depth, because of the difficulty in getting full penetration. This difficulty in getting full penetration does not affect the surface foot, and the per cent of moisture added from an irrigation continues to increase with an increase in the moisture equivalent.
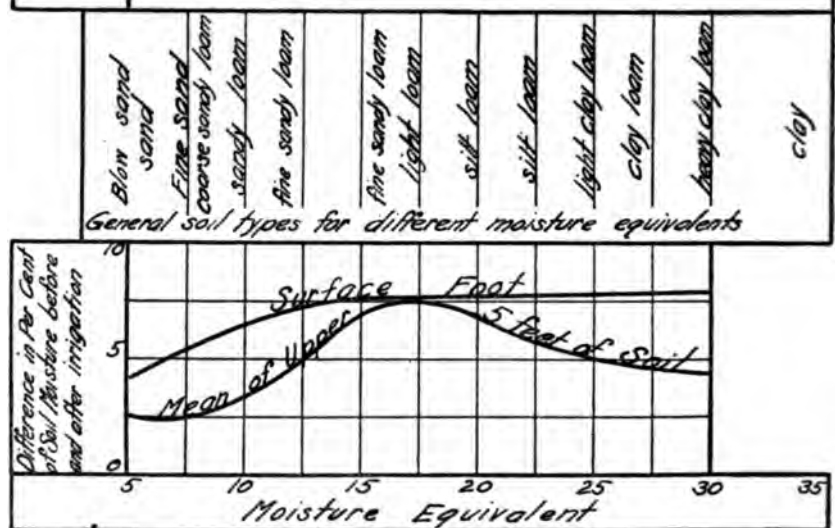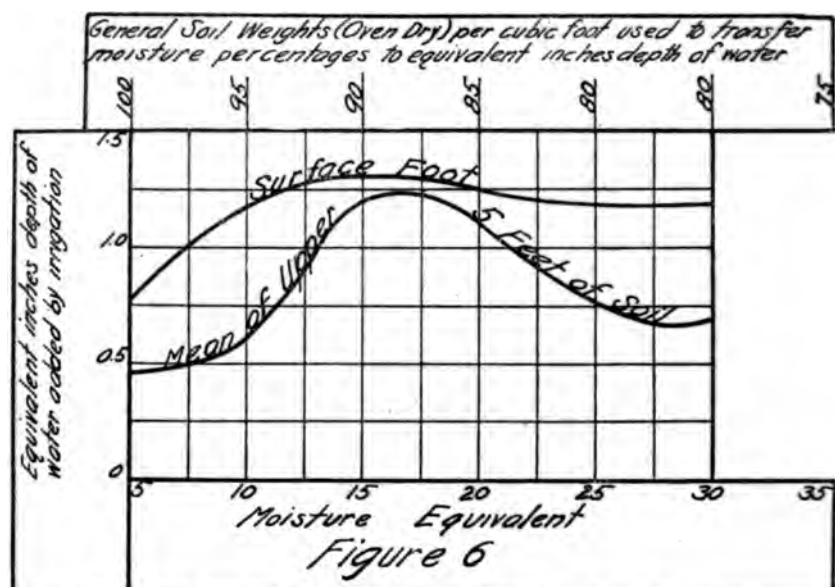
FIG. 5. RELATION OF PER CENT OF MOISTURE ADDED BY IRRIGATION UNDER FIELD CON-
DITIONS AND THE MOISTURE EQUIVALENTS

FIG. 6. RELATION OF EQUIVALENT INCHES DEPTH OF WATER RETAINED PER FOOT DEPTH
OF SOIL FROM AN IRRIGATION AND THE MOISTURE EQUIVALENT

In figure 6 the curves shown are similar to those in figure 5, the vertical ordinates in figure 6 being equivalent inches depth of water per foot depth of soil instead of soil moisture percentages as in figure 5. The general soil weights by which figure 5 is converted to figure 6 are shown at the top of the figure.

In addition the general soil types corresponding to the different values of the moisture equivalent have been written between figures 5 and 6 for convenience in reference.

In considering figures 3 to 6 the same statements regarding numerical accuracy will, of course, apply as were made regarding figures 1 and 2 from which they are derived. Caution should be used in applying figures 5 and 6, as further data will probably change the location of these curves. The general form of these curves is in agreement with general observations under field conditions and, in the author's opinion, they represent the nature of the relation of the moisture capacity of soils under field conditions to the soil texture. Further investigations might change the numerical values of points on such curves rather than their general form.

The form of the curves shown in figure 6 is in accord with general conditions of irrigation practice. Coarse soils, such as those having moisture equivalents of less than 10, are able to retain only limited amounts of water and consequently even where of good depth require frequent irrigations. In order to prevent excessive deep percolation losses on such soils the methods of irrigation must be adapted to covering them quickly so that the amount absorbed will not materially exceed the depth of water they are able to retain. On such types frequent irrigations are usually required, alfalfa generally receiving from two to three irrigations per cutting.

Soils having moisture equivalents of from 15 to 18 where of good depth are the most favorable of any in their moisture properties under irrigation. These combine a large moisture-storing capacity with a rate of absorption which permits them to be irrigated by such methods as will enable the moisture capacity to be utilized without excessive deep percolation losses. Such soils will usually carry alfalfa on one irrigation per cutting. Where properly handled very good economy in the utilization of irrigation supplies can be secured on these soils; where not properly prepared or where the water is not carefully handled they are sufficiently light to permit large percolation losses with consequent low efficiency in the application of water.

The heavy soils absorb water so slowly that it is usually not practicable to utilize the moisture capacity to the 5-foot depth without permitting the water to run sufficiently long so that other injuries such as scalding of the crop will occur. Frequently on such soils the moisture penetration will not exceed 2 feet in depth, with the result that frequent light applications must be made. This condition may cause a lower efficiency in the use of water and of the labor of its application than on soils of somewhat lighter texture.

The data presented, while subject to the limitations of extent and of accuracy covered in the above discussion, are thought to warrant the following general conclusions. All conclusions are limited in their application to field conditions under actual irrigation practice.

1. There is a fairly consistent relationship between the moisture equivalent and the various moisture properties of soils, which appears to offer promise of usefulness in determining moisture properties and probable irrigation practice of soils whose irrigation is contemplated, particularly as to the probable depth of water which will be retained from an irrigation with its effect on the depth to be applied and the necessary frequency of application.

2. The data presented, while indicating the general nature of the relationship of soil moisture capacity and soil texture, are not sufficient to fix the numerical values of such relationships except in a very general way.

3. The relationship of the soil-moisture properties to the moisture equivalent does not appear to be linear except in the case of the wilting of the crop.

4. The maximum depth of water per foot depth of soil which can be retained under favorable conditions for the upper 5 feet of soil is about 1.25 inches, which indicates that depths of single irrigation in excess of 6 to 8 inches, even under favorable soil conditions, will not be retained in the upper five or six feet of soil. This conclusion is in accord with the results of general field observations from many sources.

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THE RELATION OF CERTAIN ACIDIC TO BASIC CONSTITUENTS OF THE SOIL AFFECTED BY AMMONIUM SULFATE AND NITRATE OF SODA[1]

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INTRODUCTION

It is well known that the continuous use of ammonium sulfate produces an acid condition in the soil, whereas nitrate of soda tends toward the production of an alkaline condition.

The former salt is referred to as a physiologically acid salt while the latter is physiologically alkaline. These two terms are significant phrases; the first indicates, for example, that ammonium sulfate is active in producing a condition in the soil which affects the plant from a physiological standpoint in much the same manner as does an acid.

An acid condition in soils is comparable to the condition of acidosis in the animal organism, and reflects a change in the position of equilibrium between acids and bases. A study of this relation of acids to bases should be of value in determining the cause of this acid condition resulting from the use of ammonium sulfate.

Perhaps the more reasonable theory has been that soil acidity resulting from the use of ammonium sulfate has been due to the relatively more extensive removal of the cation, which first undergoes nitrification, and then possible absorption by the plant. This leaves an excess of acid due to the sulfate radical; and, in case the NO_3 radical has not been removed by the plant, two acid radicals result.

These acid radicals react with bases, such as calcium and magnesium carbonates. These carbonates, which may be thought of as "buffer salts" or stabilizers of the soil reaction, may soon be inadequate to function in that capacity; they will have been neutralized by the sulfate or nitrate radical and the product either slowly leached away or retained in the soil as inactive constituents. This leaves the weaker bases, such as iron and aluminum, to act as preservers of neutrality of the soil solution. The salts of these are easily hydrolized and give rise to an acid condition. Inasmuch as the bases which are active in this reaction may be considered to be calcium, magnesium,

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[1] Contribution No. 263 from the Agricultural Experiment Station of the Rhode Island State College.

iron and aluminum, these are the ones upon which most of this work is centered.

The literature on this subject has been reviewed so frequently that a repetition does not seem necessary. It appears more direct to present the experimental data secured, with such brief references to the literature as bear directly upon the problem.

SOILS

The soils on which this work was done were sampled in June, 1916, to the depth of 7 to 8 inches from permanent Rhode Island plats, which for the past twenty-five years have had like treatment, except that one series has been supplied with nitrogen in nitrate of soda while the other has received its supply in ammonium sulfate. The same comparison has existed with and without the addition of lime.

THE BASE AND ACID RETENTION OF THE SOIL

The "lime requirement" of these soils as indicated by the Veitch (12, p. 661) and ammonia (9) methods was as follows:

PLAT NUMBER	TREATMENT	CaO PER 2,000,000 POUNDS OF SOIL	
		Veitch method	Ammonia method
		lbs.	lbs.
23	Unlimed, (NH₄)₂SO₄	8,700	6,900
25	Limed, (NH₄)₂SO₄	5,800	5,200
27	Unlimed, NaNO₃	8,100	5,500
29	Limed, NaNO₃	4,800	3,700

References (5), (6), (7) and (8) of other papers from the Rhode Island Station will serve to extend the reader's acquaintance with the plats which are now again under consideration.

Although judging from the amount of ammonia retained by the soil from plat 29 after being brought to dryness over a hot-water bath, its lime requirement is 3700 pounds; yet a maximum growth of lettuce, a plant which is very sensitive to soil acidity, was obtained when the lime requirement had been reduced by liming only to 2800 pounds. It would appear, therefore, that for practical indications of the need for lime, too much ammonia was retained by the soil from plat 29. It is doubtful if any considerable amount was retained physically, for carbon black failed to exercise such retention under the conditions of the method.

The presence in the soil not only of acidic constituents but also of considerable amounts of basic iron and aluminum compounds suggested that the retention of acids might also be of importance. It was recognized that

these basic compounds are quite insoluble and inactive, and that if any combination were formed with a volatile acid, it would be quite unstable. No surprise was occasioned, therefore, when it was found that acetic acid was not retained under the following conditions: To 25 gm. of soil from plat 23 were added 75 cc. of normal acetic acid and the mixture digested at 35 to 40°C. for 1½ hours, after which distillation was carried out under reduced pressure at 82 to 90°C. until dryness was nearly attained, when 50 cc. of water was added and the process repeated.

When a non-volatile acid, however, was added the results were different, as shown by the following method: 10-gm. lots of soil were treated with 25, 50, 75 and 100 cc., respectively, of $0.2N$ H_3PO_4 and agitated for 70 hours. After this the solutions were made up to 200 cc., filtered off, and aliquots titrated with KOH and phenolphthalein. The maximum absorption by soils from plats 23 and 29 was found to be about alike and equivalent to 21,500 pounds of phosphoric oxide per 2,000,000 pounds of soil.

The foregoing demonstrates what a large basic effect the soil may exert under laboratory conditions. It must be recognized that even under certain natural conditions these basic compounds have some effect in counteracting acidic compounds, and to that extent any method of determining lime requirements which prevents the basic materials from exerting any effect is liable to overestimate the practical needs, as has already been shown to be the case with the growth of lettuce in relation to determinations of lime requirement by the use of ammonium hydroxide or calcium hydroxide, both of which substances would prevent all opportunity for the basic material in the soil to enter into the reaction.

It was shown by the following that a neutral salt may modify materially the lime requirements as determined by the ammonia method. A $0.07N$ solution with a pH of 7 by the Sörensen method was prepared by mixing mono- and di-ammonium phosphate. Before making the determination of lime requirements as usual by the ammonia method, 10 cc. of the phosphate solution were triturated lightly and occasionally with the soil by means of a pestle for an hour. The average depression in ammonia retention, caused by the addition of the ammonium phosphate, was 27 per cent, the variation with the four soils not being very marked. It is practically the mono-ammonium phosphate which is stable under the conditions of the method.

EXCHANGE OF BASES

One hundred and fifty grams of soil were shaken by inverting ten times at each interval of 15 minutes for a period of 3 hours with 500 cc. of a solution of normal KCl. These mixtures stood over night and were then filtered. The reaction of the solutions was as follows:

Plat

Unlimed, (NH₄)₂SO₄..Acid to methyl orange
Limed, (NH₄)₂SO₄..Slightly acid to methyl red
Unlimed, NaNO₃..Slightly acid to methyl red
Limed, NaNO₃..Neutral to neutral red

By titration of 50 cc. (equivalent to 15 gm. of soil) with phenolphthalein as indicator, the following was obtained:

PLAT	END POINT 0.01N NaOH	
	Cold	Hot
	cc.	cc.
Unlimed, (NH₄)₂SO₄.	21.0	24.1
Limed, (NH₄)₂SO₄.	2.3	3.0
Unlimed, NaNO₃.	4.7	5.4
Limed, NaNO₃.	0.6	0.8

During the titration of the extract from the unlimed sulfate of ammonia plat, a precipitate formed which resembled aluminum hydroxide. Ammonium hydroxide was added to an aliquot of the extract from each of the soils, but the precipitate formed only in the above-mentioned case. This precipitate was ignited and weighed. In an aliquot equivalent to 90 gm. of soil the weight of the precipitate was 0.0063 gm. If the acid condition revealed by titration with sodium hydroxide is attributed to the acidity of a hydrolyzed aluminum salt, and the weight of aluminum oxide is calculated equivalent to the sodium hydroxide used in the titration, we obtain, in an aliquot equivalent to 90 gm., a weight of 0.0066 gm. The weight obtained by direct precipitation and weighing was, as indicated above, 0.0063 gm. On this assumption practically all the titrated acidity may be accounted for as due to hydrolysis of an aluminum salt. G. Daikuhara (3) has demonstrated that in certain Japanese soils the acidity is proportional to the amount of aluminum present.

The analyses of the solutions follow:

Bases brought into solution from 90 gm. of soil by KCl

PLAT	Al₂O₃	CaO	MgO	TOTAL EXCHANGE OF BASES IN TERMS OF CaO
	gm.	gm.	gm.	gm.
Unlimed, (NH₄)₂SO₄.	0.0063	0.0296	0.0026	0.0426
Limed, (NH₄)₂SO₄.		0.0666	0.0113	0.0780
Unlimed, NaNO₃.		0.0393	0.0028	0.0421
Limed, NaNO₃.		0.1030	0.0187	0.1217

An exchange of base takes place when the four soils are treated with a solution of neutral potassium chloride. Potassium replaces calcium and magnesium from the limed plats while in the unlimed plat receiving ammonium sulfate, aluminum also is replaced. Considerable sulfur was present also in the latter instance. Ruprecht and Morse (11) report a similar exchange resulting from the use of ammonium sulfate.

EXTRACTION WITH 0.2 N HCl

Two hundred grams of soil were digested with 2 liters of 0.2N HCl at room temperature. The mixture was shaken by inverting ten times every hour for the first 8 hours. It was allowed to remain over night, filtered, and washed free of acid. The solution was evaporated, silicon hydroxide dehydrated, and the solution for analysis made in hydrochloric acid. The analysis of this solution follows:

PLAT	$Al_2O_3 Fe_2O_3$ $P_2O_5 (TiO_2)$	Fe_2O_3 — (TiO_2)	Al_2O_3	P_2O_5	CaO	MgO
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Unlimed, $(NH_4)_2SO_4$	1.190	0.1515	0.9882	0.0503	0.0125	0.0009
Limed, $(NH_4)_2SO_4$	1.232	0.1224	1.0657	0.0439	0.0556	0.0057
Unlimed, $NaNO_3$	1.168	0.1120	1.0041	0.0519	0.0212	0.0021
*Limed, $NaNO_3$	1.229	0.0975	1.0805	0.0510	0.1091	0.0092

* This soil was the only one giving a reaction for manganese.

If it is true that a lack of calcium and magnesium imposes on iron and aluminum the responsibility of maintaining the neutrality of the soil solution, then on the unlimed as compared with the limed soil there should exist a very different relation between the two pairs of bases. This relation is shown by the following:

PLAT	$\dfrac{Al_2O_3 + Fe_2O_3}{CaO + MgO}$
Unlimed, $(NH_4)_2SO_4$	85.0
Limed, $(NH_4)_2SO_4$	19.4
Unlimed, $NaNO_3$	47.9
Limed, $NaNO_3$	10.0

A great variation does exist. It is impossible to estimate the actual significance of this relation but it harmonizes well with the belief that a great deal of the condition referred to as soil acidity is due to the hydrolysis of salts in which weak bases, such as iron and aluminum, are undertaking the responsibility preferably assumed by the stronger bases, calcium and magnesium.

EXTRACTION WITH NH₄OH

After the acid extraction, the soil was digested with 2 liters of 4 per cent NH₄OH by a procedure similar to that employed in making the acid extract. A clear solution was obtained by re-filtering several times through the soil mass on a large filter. The extract was boiled with ammonium carbonate and filtered. The following is the percentage analysis of this extract.

PLAT	Fe₂O₃	Al₂O₃	P₂O₅	CaO	MgO	SiO₂
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Unlimed, (NH₄)₂SO₄.............	0.1366	0.0641	0.0857	0.0122	0.0007	0.0356
Limed, (NH₄)₂SO₄.......:........	0.1354	0.0900	0.0853	0.0086	0.0009	0.0321
Unlimed, NaNO₃...............	0.1571	0.1156	0.0984	0.0107	0.0009	0.0362
Limed, NaNO₃.................	0.1472	0.1356	0.0892	0.0142	0.0007	0.0435

In the analysis of this ammonia extract it appears that the per cent of phosphorus was not materially different on the several plats; this same observation holds true with silicon.

It is interesting to observe again the relation between the stronger and the weaker bases.

PLAT	$\dfrac{Fe_2O_3 + Al_2O_3}{CaO + MgO}$
Unlimed, (NH₄)₂SO₄..	15.5
Limed, (NH₄)₂SO₄..	23.7
Unlimed, NaNO₃...	23.5
Limed, NaNO₃...	18.9

The relations here do not show great variation as compared with those obtained from the acid extract. Only a small difference appears between the very acid soil and the nearly neutral soil. This would seem to indicate that the great difference existing in these soils is not to be attributed to an unsatisfactory relation of these bases in mainly organic combination but would indicate a disturbance in their relation in the inorganic system.

HYDROGEN ION CONCENTRATION

The hydrogen ion concentration in case of these soils has been measured by the colorimetric method[2] and found to be as follows:

[2] Essentially that employed by Gillespie (4) using the phthalate "buffer" mixtures (1, 2) and sulphonphthalein indicators.

PLAT	pH	(H')
Unlimed, $(NH_4)_2SO_4$	4.0	1×10^{-4}
Limed, $(NH_4)_2SO_4$	5.3	5×10^{-6}
Unlimed, $NaNO_3$	5.2	6.3×10^{-6}
Limed, $NaNO_3$	6.0	1×10^{-6}

It is seen that the concentration of hydrogen ions in these soils shows marked differences, ranging from an acidity of $0.0001N$ in the case of the unlimed ammonium sulfate plat to $0.000001N$ in the limed sodium nitrate plat, or a difference of a hundredfold in the intensity of reaction.

The concentration of hydrogen ions indicated by a pH of 4.0 with the unlimed sulfate of ammonia plat is a close approximation to the concentration of hydrogen ions which is developed by aluminum salts dissociating through a wide range of concentrations. If the same as the chloride, aluminum salts are about one-third dissociated (10); that is to say, in a solution of an aluminum salt, the acidity in terms of hydrogen ions is not the same as that developed by a mineral acid of the same normality, but is much less. Calculation of the acidity of an aluminum salt based upon titration is similar to that of a free acid since the hydrolysis of the salt proceeds with the titration.

The relation of acidity of $Al_2(SO_4)_3$ in terms of normality and of concentration of hydrogen ions is as follows:

NORMALITY	pH	(H')	NORMALITY IN TERMS OF (H')
$0.100 N$	3.4	4×10^{-4}	$0.0004 N$
$0.010 N$	3.7	2×10^{-4}	$0.0002 N$
$0.001 N$	4.0	1×10^{-4}	$0.0001 N$

In other words, a hundredfold increase in concentration based upon normality produces only a fourfold increase in concentration of hydrogen ions.

The concentration of hydrogen ions in the most acid soil is very close to that developed by these aluminum salts in solution, and experiment has shown that relatively large amounts of these salts can be added to a buffer solution without material change in the concentration of hydrogen ions; but the soil solution is a buffer solution containing phosphates and carbonates and so it appears that whatever the concentration of aluminum salts in the soil may be, they can never affect the concentration of hydrogen ions beyond a certain point, being held in check, as they are, first, by the dissociation constant; and second, by the buffer effect of phosphates and carbonates. This point of maximum concentration of hydrogen ions resulting from this equilibrium is believed to be expressed within narrow limits by a pH of 4.

SUMMARY

Great variation in acidity exists between soils receiving nitrogen in nitrate of soda as compared with those which receive it in sulfate of ammonia.

The soil, being a mixture in part of more or less inactive basic as well as acidic constituents, cannot have its "acidity" correctly measured by reagents which react only with the acidic ingredients.

Digestion with hydrochloric acid showed that great variation existed between the quotient obtained by dividing the oxides of aluminum and iron by those of calcium and magnesium. It ranged from 85 in the case of the unlimed soil receiving sulfate of ammonia to 10 where lime and nitrate of soda were employed.

A subsequent extract with ammonium hydroxide showed no great variation in this ratio of weak to strong bases.

An exchange of bases, produced by treatment of the soil with a solution of potassium chloride, showed a marked degree of reserve acidity from the unlimed soil receiving sulfate of ammonia. The acidity, so developed, showed a correlation to the acidity of an aluminum salt equivalent in amount to the weight of aluminum which was contained in the solution obtained by shaking with potassium chloride.

In the acid unlimed soils, iron and aluminum partially took the places occupied by calcium and magnesium in the limed soils.

The soil solution acts as a buffer solution containing phosphates and carbonates.

The concentration of hydrogen ions in the soil solution from the unlimed plat receiving sulfate of ammonia is very similar to that produced by the addition of even quite large amounts of aluminum salts to buffer solutions.

CONCLUSIONS

The "acidity" in a soil caused by long-continued use of ammonium sulfate is the result of a change in the ratio of acids to bases. The position normally occupied by the stronger bases, such as calcium and magnesium, has been taken by weaker bases, such as iron and aluminum. The neutrality of the soil solution can no longer be maintained since salts of these weak bases dissociate. Free acid resulting from this dissociation is accompanied by a definite concentration of hydrogen ions.

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CALCIUM AND MAGNESIUM CONTENT OF VIRGIN AND CULTIVATED SOILS

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INTRODUCTORY

The soil samples discussed in this paper were taken subsequent to the reconnoissance soil survey of Ohio, and although only a minority of the soil types of the state are represented by the samples which have been secured up to the present, the analytical work projected upon these has been completed and it seems desirable to make at least a partial report at this time. The relationships existing between total and fifth normal nitric acid soluble calcium and magnesium, carbonate content and reaction of the soil in those cases where both virgin and cultivated samples of the same soil type were taken in close proximity, have been considered.

SAMPLES STUDIED

Samples from the two depths 0 to 7 inches and 7 to 15 inches, designated "a" and "b," respectively, in the system of laboratory numbers, were prepared for analysis in the manner prescribed by the Association of Official Agricultural Chemists (3). The sampling was done under the supervision of E. R. Allen, formerly soil technologist at this station, to whom we are indebted for the description of individual soils, and for other aid.

ANALYTICAL METHODS

Total calcium and magnesium were determined by standard methods following a sodium peroxide fusion.

Fifth normal nitric-acid-soluble calcium and magnesium were determined in aliquots of a solution prepared by digesting 220 gm. of the air-dried and prepared sample with 2200 cc. of nitric acid of such strength that the acid in contact with the soil, after 5 hours' digestion at room temperature with shaking every half hour, should be exactly fifth normal as determined by titration of a boiled and cooled aliquot with standard sodium hydroxide and phenolphthalein in a preliminary experiment. At the end of the period prescribed, the solutions were filtered as rapidly as possible on large paper filters.

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Carbonate was determined by the modified Marr method (3), titrating the residual barium hydroxide (5).

The qualitative tests for reaction were applied to the air-dried and prepared samples; Azolitmin (blue) and red litmus paper were doubled and pressed into a gash cut in a mass of the moistened soil and observed after one-half hour; the Veitch test was conducted exactly as originally described (8), save only that the extract was in every case filtered before being concentrated. The details of the Truog test were carefully followed as directed by the originator (7). All these tests were read as closely as possible, and in cases where the indications obtained were doubtful, the work was duplicated.

SIGNIFICANCE OF FIFTH NORMAL ACID SOLUBLE CALCIUM AND MAGNESIUM

In a former publication (2), the writers have presented data indicating that the fifth normal nitric acid soluble calcium and magnesium of the soil, exclusive of carbonates, approximates what may be termed the more active part of the soil's supply of the basic elements; in other words, the portion which is in combination with those constituents of the soil, whose residual capacity for absorption of a base (calcium) is indicated with more or less exactness by the term "lime requirement."

Shorey, Fry and Hazen (6) employed a method of leaching the soil with 2 per cent hydrochloric acid for the determination of that part of the soil's supply of calcium which may be present as calcium sulfate, calcium carbonate and in combination with humus; the calcium in the form of carbonate and sulfate can be calculated from other data, and any excess is supposed to represent calcium in combination with humus.

While it is doubtless a fact that a portion of the calcium and magnesium, other than carbonate, which is soluble in dilute acids is connected in some way with the organic matter of the soil, the writers are of the opinion that a more important part, in many soils, is present as very easily decomposable silicates or alumino-silicates, from which the bases are removed by acid much more dilute than 2 per cent hydrochloric acid. The data leading to this belief have been discussed (2).

It is certain that to assume all the calcium which is soluble in hydrochloric acid of 2 per cent or less strength, and not accounted for by the presence of carbonate or sulfate, to be in combination with organic matter will lead to unexpected results in some instances. An example which may be cited is the Spencer silt loam discussed by Shorey and his co-workers, the lower subsoil of which contains by far the largest percentage of calcium oxide with humus.

Data which would serve as a basis for an estimate of that part of the fifth normal nitric acid soluble calcium which is derived from calcium sulfate in the soils discussed in this paper are lacking. The omission is not considered very important, for the following reasons:

1. Shorey and associates in no case found any considerable quantity of calcium in the dilute acid extract which could be considered to be derived from calcium sulfate; the maximum amount was 0.02 per cent CaO on the basis of the sample, and in the majority of cases only a trace or none is reported.

2. The occurrence of any considerable amount of calcium sulfate in a normal soil of a humid region seems unlikely.

3. The neutralizing power of the soil was determined as a necessary preliminary to the fifth normal nitric acid digestion; the data thus obtained were also found useful as a check upon the accuracy of the calcium and magnesium determinations in the acid extracts.

Calcium sulfate could not affect the figures for acid consumed, therefore if the fifth normal nitric acid soluble calcium was appreciably in excess of the calcium equivalency of the acid neutralized, it could be attributed to calcium sulfate. Since no large difference was observed in this respect, it is evident that calcium sulfate was not present in sufficient amount to have any important bearing on the results.

DISCUSSION OF ANALYTICAL DATA

The analytical data for the soils studied are presented in table 1, and in addition to the contents of total and fifth normal nitric acid soluble calcium and magnesium, include data obtained from carbon-dioxide determinations, expressed as calcium carbonate content, and the reaction of the soils as determined by three well known qualitative tests. The percentages of the total calcium and magnesium contents soluble in fifth normal nitric acid are tabulated.

As a further aid to the interpretation of these analyses, the magnesium contents, both total and soluble, have been calculated to the equivalent amounts of calcium and these added to the corresponding figures for the calcium determined, calcium corresponding to the carbon dioxide found being deducted. As in the case of the two elements when considered separately, the percentages of the total amounts which are soluble have been tabulated.

The data last mentioned are considered to furnish useful information, as indicating both quantitatively and relatively the amount of the two bases together which may be considered as having combined with, and partially satisfied, the acid-reacting constituents. The bases present in the form of carbonates may be regarded as a reserve supply of the basic elements.

In a former publication from this laboratory (1), it was stated that for the Ohio soils studied it appeared to be a general rule that when total magnesium exceeds total calcium, no carbonates are present. With one or two exceptions, the rule appears to hold good for the soils discussed in this paper. A striking exception is the subsurface 33 b; here the amount of total magnesium is one and one-half times the percentage of total calcium, yet a very considerable quantity of carbonate is present. The proportion of the total mag-

nesium content of the soil soluble in 0.2N HNO$_3$ is unusually high, indicating that the carbonate in this soil is probably of dolomitic nature. It is noteworthy that the surface of the cultivated sample, number 32 b, taken near by, shows similar relations between total calcium and magnesium, and total and soluble magnesium, although in this case the soil is practically devoid of carbonate and reacts acid to tests. Samples 55 and 37 have much more total calcium than magnesium in both depths, but the presence of magnesium limestone is indicated by the considerable amount of magnesium soluble in 0.2N HNO$_3$.

. The high amounts of magnesium found in the soils of western Ohio are doubtless due to the presence of a considerable amount of dolomitic limestone in the glacial till from which these soils are derived.

In table 2 is presented in compact form a summarized comparison of the analytical data for the virgin and cultivated samples analyzed. It will be observed that of the two elements, calcium is preponderant in the soil in the majority of cases, but that for approximately half of the samples magnesium exceeds calcium in the subsoil. In no case where the percentage of calcium in the subsoil is greater than the magnesium can this relation be attributed solely to the presence of carbonate of calcium.

Although but a comparatively small number of samples are represented, the two depths of cultivated and corresponding virgin soils exhibit some differences in their content of fifth normal nitric acid soluble calcium and magnesium, which appear to be of significance in showing changes which occur in soils as the result of cultivation.

In the majority of instances the proportion of total calcium and magnesium, calcium especially, soluble in fifth normal nitric acid, is greater in the surface virgin soil as compared with the same depth of cultivated soil. When the 7 to 15-inch depth of these soils is compared, the reverse of this is seen in many cases, the subsurfaces of the virgin sample containing less of these bases than the cultivated soil.

In contrasting the data for the individual cultivated and similar virgin soils as presented in table 1, it will be observed that the relations referred to are not uniform and consistent. This is to be expected in view of the differences which are to be found in the chemical composition of soils of the same type. Neither should too much significance be attached to small variations in composition. Attention has been directed to these points by Robinson, Steinkoenig and Fry (4).

Considering all the samples together, however, the averaged results should be fairly reliable in their indications of the effect of cultivation in promoting the action of natural solvent agencies, and in producing changes in the physical conditions, both of which affect the distribution of bases in different depths of soil.

Reaction and carbonate content

By far the greater number of samples examined show mere traces of carbonate; while this may possibly have been formed in some cases by the decomposition of organic matter during the analytical procedure, or may have been merely occluded by the soil, as suggested by Shorey and associates (6), the possibility of a local occurrence of carbonate in noncalcareous soils acid to tests should be considered. This condition could be attributed to isolated particles of carbonate minerals, or to plant residues containing organic salts of calcium which changed to carbonate during the process of decay.

There does not appear to be any consistent relation between carbonate content and reaction when the carbonate is present in very slight amount; some soils which are acid to the indicators employed contain as much carbonate as others strongly alkaline to tests, while one soil which was alkaline to the tests made, contains no carbonate at all. With larger amounts of carbonate —0.04 to 0.05 per cent—however, the reaction is always alkaline to litmus and the Veitch test, while the Truog test is usually negative or at most indicates "very slight acidity," except in one case (no. 33 b).

Another relationship which calls for comment is that between the amount of carbonate and the proportion of the non-carbonate calcium and magnesium soluble in $0.2N$ HNO_3. With but a single exception (again 33 b) in all cases where any considerable amount of carbonate is present, the proportion of the total calcium and magnesium not in the form of carbonate, but soluble in $0.2N$ HNO_3, is higher than in the sample from a different depth or taken nearby of the same type but containing less carbonate. Indeed, this connection is plainly seen in the great majority of cases where the calcium carbonate content is very low—0.01 to 0.02 per cent—and would seem to indicate that even these small figures for carbonate are not without significance, for they indicate basicity in other forms than carbonate.

In the greater number of instances, the indications of the three methods for determining the reaction of the soil are in agreement; the indications of the litmus and Veitch tests are occasionally at variance. These tests are not adapted to making comparisons as to degree of acidity or alkalinity of different soils, hence all soils which reddened blue litmus paper to a noticeable extent are called acid, while all whose water extracts were even slightly alkaline after concentration are considered alkaline by the Veitch test. In many cases where the indications are at variance, the soil was probably near the border line, as both tests were rather indistinct. The Truog test appears to indicate some acidity for many soils alkaline to the other tests.

In the case of some of the soil types under consideration, there appears to be a fairly consistent relation between the indications of the Truog test and the percentage of the total calcium and magnesium soluble in fifth normal nitric acid, exclusive of carbonates.

TABLE 1

Calcium and magnesium content and reaction of Ohio soils

DESCRIPTION	NUMBER	MAGNESIUM			CALCIUM			CALCIUM AND MAGNESIUM AS CALCIUM EXCLUSIVE OF CARBONATES			CaCO₃	REACTION		
		Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble		Litmus	Truog	Vetch
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent			
Wooster loam — Cultivated	4a	0.236	0.0037	1.6	0.190	0.0360	19.0	0.576	0.0951	16.5	0.005	Acid	Medium acid	
	4b	0.269	0.0074	2.8	0.184	0.0561	30.5	0.627	0.0681	10.9		Acid	Strongly acid	
Virgin	5a	0.210	0.0067	3.2	0.253	0.0900	35.6	0.591	0.093	15.7	0.020	Acid	Medium acid	
	5b	0.228	0.0081	3.6	0.148	0.0240	16.2	0.521	0.035	6.7	0.005	Acid	Strongly acid	
Wooster loam — Cultivated	6a	0.220	0.0054	2.5	0.223	0.0310	13.9	0.583	0.038	6.5	0.005	Acid	Medium acid	
	6b	0.250	0.0127	5.1	0.227	0.0413	18.2	0.638	0.062	9.7		Acid	Medium acid	
Virgin	7a	0.258	0.0079	3.1	0.214	0.0410	19.2	0.638	0.054	8.5		Acid	Strongly acid	
	7b	0.261	0.0096	3.7	0.207	0.0478	23.1	0.625	0.053	8.5	0.028	Acid	Medium acid	
Wooster silt loam — Cultivated	8a	0.307	0.0100	3.3	0.312	0.0935	29.8	0.809	0.102	12.6	0.020	Acid	Slightly acid	
	8b	0.416	0.0180	4.3	0.283	0.0805	28.5	0.968	0.110	11.4		Acid	Strongly acid	
Virgin	9a	0.288	0.0142	4.9	0.361	0.1638	45.3	0.816	0.168	20.6	0.048	Neutral	Very slightly acid	
	9b	0.276	0.0208	7.5	0.285	0.0588	20.6	0.737	0.091	12.3	0.005	Acid	Slightly acid	

Soil	Treatment	Sample													
Cincinnati silt loam	Cultivated	19a	0.256	0.0096	3.8	0.323	0.0922	28.5	0.742	0.106	14.3	0.005	Acid	Slightly acid	Alkaline
		19b	0.410	0.0164	4.0	0.270	0.1035	38.3	0.943	0.129	13.7	0.005	Acid	Medium acid	Alkaline
	Virgin	20a	0.274	0.0110	4.0	0.290	0.1151	39.7	0.735	0.128	17.4	0.013	Acid	Slightly acid	
		20b	0.315	0.0147	4.7	0.250	0.0667	26.7	0.768	0.091	11.9		Acid	Strongly acid	
Cincinnati silt loam	Cultivated	17a	0.262	0.0156	6.0	0.298	0.0981	32.9	0.720	0.115	16.0	0.023	Acid	Slightly acid	
		17b	0.332	0.0174	5.2	0.300	0.0812	27.1	0.845	0.108	12.8	0.005	Acid	Strongly acid	
	Virgin	23a	0.265	0.0091	3.6	0.250	0.0517	20.7	0.679	0.060	8.8	0.018	Acid	Slightly acid	
		23b	0.191	0.0180	9.4	0.210	0.0547	26.1	0.522	0.083	15.9	0.005	Acid	Very strongly acid	
	Cultivated	21a	0.256	0.0100	3.9	0.335	0.0881	26.3	0.754	0.103	13.7	0.005	Acid	Very slightly acid	Alkaline
	Cultivated	21b	0.287	0.0230	8.0	0.245	0.0818	33.4	0.715	0.118	16.5	0.005	Acid	Very strongly acid	
Clermont silt loam	Virgin	22a	0.238	0.0180	7.6	0.295	0.1065	36.1	0.681	0.131	19.2	0.015	Neutral	Very slightly acid	Alkaline
	Virgin	22b	0.345	0.0240	7.0	0.285	0.0596	20.9	0.851	0.097	11.4	0.005	Acid	Very strongly acid	
Crosby silt loam	Cultivated	34a	0.269	0.0198	7.4	0.445	0.1343	30.2	0.887	0.167	18.8		Acid	Slightly acid	Alkaline
		34b	0.453	0.0442	9.8	0.430	0.0815	19.0	1.174	0.153	13.0	0.005	Acid	Very strongly acid	
	Virgin	35a	0.270	0.0257	9.5	0.440	0.0965	21.9	0.882	0.137	15.5	0.005	Acid	Medium acid	
		35b	0.416	0.0449	10.8	0.413	0.0640	15.5	1.096	0.136	12.4	0.005	Acid	Strongly acid	

TABLE 1—Continued

DESCRIPTION		NUMBER	MAGNESIUM			CALCIUM			CALCIUM AND MAGNESIUM AS CALCIUM EXCLUSIVE OF CARBONATES			CaCO₃	REACTION		
			Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble	CaCO₃	Litmus	Truog	Veitch
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent			
Lucas silt loam	Cultivated	38a	0.513	0.0355	6.9	0.593	0.1745	29.4	1.420	0.216	15.2	0.043	Alkaline	Slightly acid	Alkaline
		38b	0.789	0.0688	8.7	0.465	0.1560	33.6	1.761	0.267	15.2	0.005	Acid	Strongly acid	Alkaline
	Virgin	39a	0.553	0.0460	8.3	0.490	0.2240	45.7	1.392	0.292	21.0	0.020	Neutral	Very slightly acid	Alkaline
		39b	0.617	0.0548	8.9	0.538	0.2230	41.4	1.547	0.307	19.8	0.015	Neutral	Very slightly acid	Alkaline
Fox silt loam	Cultivated	59a	0.329	0.0200	6.1	0.415	0.1020	24.6	0.956	0.135	14.1		Acid	Medium acid	Alkaline
		59b	0.449	0.0344	7.7	0.425	0.1493	35.1	1.163	0.206	17.7		Acid	Medium acid	
	Virgin	60a	0.348	0.0385	11.1	0.498	0.1980	39.8	1.063	0.253	23.8	0.020	Neutral	Negative	Alkaline
		60b	0.496	0.0459	9.3	0.513	0.1900	37.0	1.325	0.262	19.8	0.010	Neutral	Very slightly acid	Alkaline
Maumee fine sand	Cultivated	28a	0.108	0.0029	2.7	0.320	0.0120	3.8	0.498	0.017	3.4		Acid	Very strongly acid	
		28b	0.108	0.0014	1.3	0.320	0.0027	0.8	0.498	0.005	1.0		Acid	Medium acid	
	Virgin	29a	0.200	0.0121	6.1	0.455	0.1250	27.5	0.784	0.145	18.5		Acid	Slightly acid	
		29b	0.126	0.0064	5.1	0.420	0.0790	18.8	0.627	0.090	14.4		Acid	Slightly acid	

Soil		No.													
Newton loam	Virgin	50a	0.422	0.0635	15.1	1.130	.5174	45.8	1.804	0.600	33.3	0.054	Alkaline	Negative	Alkaline
		50b	0.409	0.0645	15.8	0.870	.3632	41.8	1.537	0.463	30.2	0.015	Alkaline	Negative	Alkaline
Dunkirk fine sand	Cultivated	25a	0.240	0.0085	3.5	0.620	.0508	8.2	1.015	0.065	6.4	0.005	Neutral	Slightly acid	Alkaline
		25b	0.205	0.0027	1.3	0.485	.0162	3.3	0.822	0.021	2.5		Neutral	Medium acid	
	Virgin	26a	0.183	0.0069	3.8	0.435	.0359	8.3	0.736	0.047	6.4		Acid	Medium acid	
		26b	0.170	0.0034	2.0	0.430	.0050	1.2	0.710	0.011	1.5		Acid	Medium acid	
		45a	0.223	0.0048	2.2	0.555	.0400	7.2	0.921	0.048	5.2		Alkaline	Very slightly acid	
Dunkirk fine sand	Cultivated	45b	0.244	0.0029	1.2	0.540	.0195	3.6	0.941	0.024	2.6		Alkaline	Very slightly acid	Alkaline
	Virgin	46a	0.253	0.0039	1.5	0.590	.0215	3.6	1.006	0.028	2.8		Acid	Medium acid	
		46b	0.286	0.0034	1.2	0.663	.0160	2.4	1.133	0.022	1.9		Acid	Slightly acid	
	Cultivated	47a	0.198	0.0123	6.2	0.575	.0610	10.6	0.871	0.051	5.9	0.074	Alkaline	Negative	Alkaline
		47b	0.228	0.0044	1.9	0.690	.0265	3.8	1.059	0.033	3.1	0.015	Alkaline	Negative	Alkaline
Dunkirk fine sand	Virgin	48a	0.202	0.0086	4.3	0.585	.0194	3.3	0.917	0.034	3.7		Acid	Very strongly acid	
		48b	0.263	0.0055	2.1	0.533	.0194	3.6	0.966	0.028	2.9		Acid	Very strongly acid	
Belmore sandy loam	Cultivated	55a	0.286	0.0893	31.2	0.890	.4149	46.7	1.136	0.337	29.7	0.561	Alkaline	Negative	Alkaline
		55b	0.301	0.0298	9.9	0.685	.2507	36.6	1.117	0.241	21.5	0.149	Alkaline	Negative	Alkaline
	Virgin	56a	0.242	0.0183	7.6	0.593	.1781	30.1	0.985	0.202	20.5	0.015	Alkaline	Negative	Alkaline
		56b	0.268	0.0181	6.8	0.520	.1493	28.7	0.865	0.173	17.9	0.015	Alkaline	Very slightly acid	Alkaline

TABLE 1—Continued

DESCRIPTION	NUMBER	MAGNESIUM			CALCIUM			CALCIUM AND MAGNESIUM AS CALCIUM EXCLUSIVE OF CARBONATES			CaCO₃	REACTION		
		Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble	Total	0.2N HNO₃ soluble	Percentage of total soluble		Litmus	Truog	Veitch
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent			
Miami silty clay loam — Cultivated	30a	0.722	0.0290	4.0	0.393	0.1123	28.6	1.578	0.158	10.0	0.005	Acid	Medium acid	
	30b	0.682	0.0681	10.0	0.373	0.1555	41.7	1.493	0.266	17.8	0.005	Acid	Very strongly acid	
Virgin	31a	0.372	0.0229	6.2	0.480	0.2204	45.9	1.079	0.245	22.7	0.033	Alkaline	Slightly acid	Alkaline
	31b	0.569	0.0351	6.2	0.280	0.0790	28.2	1.213	0.133	11.0	0.010	Acid	Very strongly acid	
Miami silty clay loam — Cultivated	32a	0.381	0.0269	7.1	0.390	0.1113	28.6	1.008	0.146	14.5	0.023	Acid	Medium acid	Alkaline
	32b	0.553	0.0892	15.6	0.360	0.1689	46.9	1.259	0.304	24.2	0.030	Acid	Medium acid	
Virgin	33a	0.335	0.0298	8.9	0.475	0.1816	38.2	1.022	0.226	22.1	0.013	Acid	Medium acid	Alkaline
	33b	0.730	0.1623	22.3	0.525	0.3089	58.8	1.441	0.291	20.2	0.713	Alkaline	Slightly acid	
Brookston silty clay loam — Cultivated	36a	0.583	0.0772	13.2	0.810	0.3680	45.4	1.766	0.491	27.8	0.010	Alkaline	Very slightly acid	Alkaline
	36b	0.688	0.0984	14.3	0.840	0.3570	42.5	1.966	0.516	26.3	0.015	Alkaline	Negative	Alkaline
Virgin	37a	0.645	0.1490	23.1	1.040	0.6160	59.2	2.040	0.799	39.2	0.155	Alkaline	Negative	Alkaline
	37b	0.768	0.2051	26.7	0.985	0.5640	57.2	2.000	0.654	32.7	0.621	Alkaline	Negative	Alkaline

Soil	Treatment	Sample												
Brookston clay loam	Virgin	41a	0.549	0.0579	10.5	1.280	0.6380	49.9	2.161	0.711	32.9	0.056	Alkaline	Negative	Alkaline
		41b	0.627	0.0560	8.9	0.918	0.3500	38.1	1.940	0.432	22.3	0.025	Alkaline	Negative	Alkaline
Brookston clay	Cultivated	42a	0.420	0.0298	7.1	0.708	0.2687	37.9	1.393	0.312	22.5	0.015	Neutral	Very slightly acid	Alkaline
		42b	0.642	0.0709	11.0	0.635	0.2520	39.7	1.687	0.365	21.7	0.010	Alkaline	Very slightly acid	Alkaline
	Virgin	43a	0.636	0.0872	13.7	1.095	0.6840	62.5	1.972	0.659	33.4	0.422	Alkaline	Negative	Alkaline
		43b	0.631	0.0660	10.5	0.820	0.3950	48.2	1.853	0.499	27.0	0.013	Alkaline	Negative	Alkaline
Brookston clay	Cultivated	51a	0.694	0.0724	10.4	1.135	0.6079	52.6	2.267	0.717	31.6	0.025	Alkaline	Negative	Alkaline
		51b	0.774	0.0994	12.2	1.060	0.6288	59.3	2.114	0.572	27.1	0.549	Alkaline	Negative	Alkaline
	Virgin	52a	0.693	0.0919	13.3	1.160	0.6865	59.2	2.243	0.780	34.8	0.144	Alkaline	Negative	Alkaline
		52b	0.792	0.0922	11.7	0.975	0.4925	50.5	2.267	0.633	28.0	0.028	Alkaline	Negative	Alkaline
Newton silty clay	Cultivated	53a	0.675	0.0896	13.3	1.280	0.8822	68.9	2.276	0.926	40.7	0.260	Alkaline	Negative	Alkaline
		53b	0.769	0.0927	12.1	0.895	0.5104	57.0	2.149	0.651	30.3	0.030	Alkaline	Negative	Alkaline
	Virgin	54a	0.729	0.0853	11.7	1.180	0.7721	65.5	2.372	0.904	38.1	0.020	Alkaline	Negative	Alkaline
		54b	0.848	0.0589	6.9	0.925	0.5055	54.7	2.316	0.598	25.9	0.013	Alkaline	Negative	Alkaline
Brookston clay	Cultivated	57a	0.826	0.0773	9.4	1.025	0.5791	56.5	2.380	0.702	29.5	0.010	Alkaline	Very slightly acid	Alkaline
		57b	0.819	0.0890	10.9	0.858	0.4497	52.4	2.200	0.590	26.8	0.015	Alkaline	Negative	Alkaline
	Virgin	58a	0.697	0.0942	13.5	1.125	0.7323	65.1	2.250	0.865	38.5	0.554	Alkaline	Negative	Alkaline
		58b	0.785	0.0879	11.2	0.820	0.4428	54.0	2.104	0.580	27.6	0.020	Alkaline	Negative	Alkaline

TABLE 2

Cultivated and virgin samples of the same soil types compared

	CULTIVATED	VIRGIN
	per cent	*per cent*
Total calcium in soil exceeds total magnesium in soil................	91	87
Total calcium in subsoil exceeds total magnesium in subsoil.........	48	61
Total magnesium in subsoil is greater than total magnesium in surface soil..	78	74
0.2N HNO₃ soluble magnesium in subsoil is greater than 0.2N HNO₃ soluble magnesium in soil....................................	83	61
Percentage of total magnesium soluble is greater in subsoil than in soil..	70	48
Total calcium in soil is greater than in subsoil.....................	74	83
0.2N HNO₃ soluble calcium is greater in soil than in subsoil.........	70	83
Percentage of total calcium soluble is greater in soil than in subsoil..	57	83
Percentage of both bases soluble, exclusive of carbonates, is greater in soil than in subsoil..	74	91

* Per cent of cases in 23 samples.

SUMMARY

In this paper, the contents of total and fifth normal nitric acid soluble calcium and magnesium, carbonate and reaction of both virgin and cultivated soils from 23 locations in Ohio are discussed.

The data indicate that in most virgin soils there is a concentration of readily soluble calcium and magnesium at the surface. With cultivated soils, however, the subsoil is usually better supplied with these elements than the surface soil.

Certain interesting relationships between the proportion of the total calcium and magnesium which are soluble, and carbonate content and reaction are pointed out. These may be summarized in the statement that when the proportion of the total bases (calcium and magnesium together) which is soluble, is high, the soil is very likely to contain more carbonate or to be more basic to tests than is the case with similar soils having a less proportion of these bases soluble. Moreover, this is true for soils containing very small amounts, as well as those better supplied with carbonates.

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THE LIBERATION OF NATIVE SOIL POTASSIUM INDUCED BY DIFFERENT CALCIC AND MAGNESIC MATERIALS, AS MEASURED BY LYSIMETER LEACHINGS

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Ultimate analyses of the heavier types of soils usually demonstrate the occurrence of an abundance of potassium. Parallel water and acid extractions further demonstrate that such stores of potassium are usually retained with great tenacity. However, laboratory studies show that an added solution of a neutral salt of one alkali, or alkali-earth element, may effect the liberation of an approximate equivalence of another element locked within the soil, if contact between soil and salt solution is maintained for an appreciable period. After contact with soils, neutral solutions of calcium nitrate, calcium sulfate, calcium chloride, calcium acetate, and similar calcium salts are found to contain salts of magnesium and potassium, particularly where protracted contact is permitted before the soil suspension is filtered from the extracting solution. While it is seldom, if ever, contended that the amounts of soluble neutral salts added to the soil as components of fertilizer materials, would effect a liberation of potassium sufficient to care for maximum crop production, it has nevertheless been taught that this basic exchange may be of such magnitude as to be considered of practical value. It is obvious, however, that in humid regions field conditions would not be encountered, ordinarily, where the active mass of the free soil-water solution forcing an exchange would attain such freedom of movement and intensity of action as would be maintained under laboratory conditions.

In the application of the principle of basic exchange under field conditions, it has been assumed that carbonate of lime will function as do the more soluble neutral salts; for, among the benefits advanced, as possibly accruing from the use of lime, we find frequent mention of the supposed liberation of potassium to the growing plant. Granting that in practice the soluble neutral salts of calcium may liberate appreciable amounts of potassium, it seems hardly conservative to deduce that the same function may be inherent with the relatively weak solution of $CaCO_3$ in the carbonated water of the soil. It would seem quite possible that the speed of the hydrolysis of potassium minerals, effected by the carbonated water of the soil, may reach the possible maximum under the existing conditions of surface and temperature without any acceleration to be accredited to calcium carbonate carried in solution by the soil water.

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Indeed, the results offered by Plummer (27) would seem to establish this point in so far as the original potassium-bearing minerals are concerned. However, we do not know that the solubilities of the crushed lump minerals are absolutely comparable to those of residual fragments of the same minerals, when the latter have been included in the soil mass for ages.

The work of Parker (25) indicates that, in their liberative action, soluble neutral salts of calcium function in a different manner (or at least with much greater intensity) from that to be expected from applications of either burnt lime or ground limestone. According to Parker's interpretation of the supposed base-exchange reaction in soils, the calcium-ions of a neutral salt are absorbed by the soil and, in effect, thereby generate HCl, which then extracts potassium from the soil's potassic complexes. The addition of NaOH, in amounts empirically determined as sufficient to neutralize the acid thus engendered, will inhibit the extraction of potassium effected by the engendered acid.

However, were carbonate of lime added to a soil, the calcium-ion would be absorbed and the weak CO_3-ion liberated; and, as differing from the generation of HCl incident to the absorption of the basic ion of a neutral chloride salt, this might result in a loss of CO_2 to the atmosphere, through the subsequent dissociation of H_2CO_3; or, on the other hand, it might result in a more concentrated CO_2 content in the soil-water solution effective for bathing the potassic minerals, or complexes derivative therefrom. The potassium thus coming into solution would be a measure of the readiness of the potassic complexes to undergo hydrolysis. In principle, but in extent possibly governed by surface of the applied amendment, the same reasoning would apply to both burnt and hydrated lime, as well as to limestone. For the work reported by MacIntire(21) has demonstrated that a surface application of finely divided burnt or hydrated lime will revert to the carbonate within an exposure period of four or five days prior to inworking.

LABORATORY AND POT STUDIES

The problem of augmenting available potassium has been studied in both laboratory and pot experimentation. Gaither (12) concluded from his studies at the Ohio Agricultural Experiment Station that little, if any, potassium is made available by the action of lime on soils. Gaither also gave analyses of wheat grown upon limed plats at the Ohio station. These analyses show that the application of lime has resulted in a depression in the potash content of the wheat ash. This depressive tendency of lime was likewise demonstrated by the analyses of wheat, grain and straw, from the limed plats of the Pennsylvania Agricultural Experiment Station, reported by MacIntire (19). Lyon and Bizzell (18) found no potassium enrichment in the ash of plants from limed soil, when averaging the potassium content of maize, oats, wheat, and grasses grown in their lysimeters during a period of four years.

In the study of the effect of additions of lime upon soil potassium, Gaither (12) was unable to obtain conclusive data in making water extractions. He then utilized N/5 HNO₃, the extracting period extending over 5 hours. In this case Volusia silt loam was used. The soil had been treated with varying amounts of lime, equivalent to rates of 2, 4, 6, 8, 10, 12, 15, 18, 20, and 25 tons of CaO per acre, after which two crops of alfalfa were grown. Just prior to the harvesting of the second crop of alfalfa, the soil samples were taken for extraction. The average of the two extractions from the checks gave 0.0097 per cent K_2O, while the average of the 10 lime-treated pots gave 0.0094 per cent. From these data Gaither concluded, "The theory held, 'that lime added to the soil increases the amount of available potash in the soil,' is either erroneous or requires more positive proof than has heretofore been obtained before it can be accepted."

Morse and Curry (24) concluded that while some liberation of potassium may result from lime treatment, the liberated potassium is again absorbed by the soil. Patton (26), in a résumé of earlier work, cites the experiments of Treutler, demonstrating that gypsum will yield potash to soil percolates. Storer (31) writes that investigations "have shown that gypsum exerts a powerful action in setting free potash, which has been absorbed and fixed by the earth, that is to say, by double silicates in the earth." Storer also stresses the importance of his contention, that not only is it true that "gypsum sets free potash (as well as magnesia) for the use of the crop, but it causes potash to be transferred to the lower layers of the soil so that the roots can everywhere find a store of it." Storer offered data secured by Boussingault from analyses of two crops of clover. These data show an increased potash content in each crop of clover where gypsum was applied. It is further stated that "numerous trials of leachings from loams, in great variety, with a solution of gypsum have shown that much more potash, magnesia and soda can be extracted by this solvent than by mere water."

Bradley (3) mixed three soils with 1 per cent each of CaO and $CaSO_4$, separately, and maintained 20 to 25 per cent moist contact for 6 weeks. At the end of this period the soils were leached with distilled water and the extracted potash determined. Average parts per million of 19.9, 26.0, and 34.1 for the blank, CaO and $CaSO_4$ extractions, respectively, were obtained. However, in a 24-hour-contact study, with occasional shaking and using 50 gm. of soil and 1 gm. of CaO or 1 gm. of $CaSO_4$, followed by filtration through a Pasteur-Chamberland filter, an increase in soluble potash was found only in the case of the $CaSO_4$ treatment; the CaO treatment having effected a depression in the amount of potash as compared with that obtained in distilled water blanks. The same findings were obtained in using pegmatite for similar extractions. In stating that gypsum has been very beneficial to soils of western Oregon, Bradley (3) states, "Tests on a number of these soils with lime and gypsum under different conditions indicate that gypsum acts as an indirect fertilizer, while lime does not." No reference is made to the possible beneficial action

to be derived from the sulfur content of the gypsum. Bradley gives some interesting data upon the question of the long-continued use of gypsum upon the solubility of the residual potash. A soil which had received gypsum treatments over a period of 25 years was compared with the same soil in an adjoining field which had not received such treatment. The data are here given:

	UNPLASTERED SOIL	PLASTERED SOIL
	per cent	*per cent*
Potash soluble in HCl, 1.115..............................	0.202	0.268
Potash soluble in HNO₃, N/5..............................	0.050	0.069

From these data it would appear that the prolonged gypsum treatment had appreciably enhanced the solubility of the residual potash.

McMiller (23), using five soils and applying to each, separate applications of 1 per cent CaO and CaSO₄, after 3 months found an increase of 82 per cent in the water-soluble potash. In studies with granite-derived soils and their separates, Dumont (9) found that heavy additions of calcium sulfate and long-continued moist contact were instrumental in liberating potash; and that the liberation increased coincident with the decrease in fineness of the separates. Working with New York soils, Tressler (32) found that in some instances potassium was liberated from the more insoluble forms by the use of soluble lime salts. In the case of the relatively young glacial soils used by Tressler, the potash-bearing materials were probably, in the main, undisintegrated ground-up minerals, rather than the hydrolyzed residues characteristic of soils formed *in situ*. Andre (2) found that the solubility of the potash in microline, a potassic mineral commonly found in soils, was increased by treatment with CaSO₄.

Fraps (11), in pot studies involving particularly a study of the acid-soluble potassium and that taken up by plants, came to the conclusion that additions of CaCO₃ and CaSO₄ did not effect any appreciable liberation of soil potash, nor was there induced any increase in the potash assimilated by the several crops. Additions of CaCO₃ were found not to have increased the amount of active potash present in the several soils at the end of the period of experimentation.

In making 17 distilled water extractions from a number of the fertilizer plats at the Pennsylvania station, under and following oats, during a period between May 13 and September 30, Brown and MacIntire (5) found less potassium in the extracts from the limed plats than from those unlimed, after 29 years of liming. The average of the 17 extractions from the limed and manured plat no. 22 amounted to but 6.85 parts per million of potassium as compared with 8.30 parts per million as an average from plat 16, which received manure alone. Again, the burnt-lime plat no. 23 gave an average potassium concentration of only 6.0 parts per million as against 6.5 for the

check plat no. 24. Thus, while it might be assumed that lime may have induced the liberation of potash initially, the long-continued occurrence of the accumulated supply of carbonate of lime ultimately decreased the extent of potash liberation, or else enhanced the soil's tendency to reabsorb any potassium released from siliceous combination. In this connection it might be noted that about one-third of the lime accumulated over a period of 30 years was held as silicates, or other forms than CaCO₃, although the untreated check plats now show slight, and in some cases no lime absorption, as measured by the Veitch (33) method studies of Gardner and Brown (13). The residual K₂O found in these soils after 30 years of liming will be considered in another part of this treatise.

In considering the plant as an indicator of the availability of potash after treatments of lime, the results of Fraps (10), Gaither (12), Lyon and Bizzell (18) and those cited by Storer (31) suggest the consideration of the potash composition of crops grown on the lime plats of the Pennsylvania station. Such data are available. In 1910 MacIntire (19) studied the composition of the wheat crop from nine of the plats of tier 2. Liming produced no effect upon the potassium content of the grain. However, in the case of wheat straw, a considerable decrease was effected as a result of liming after a period of 29 years. The straw from the plat which had received 2 tons of burnt lime every 4 years analyzed but 0.94 per cent K₂O, as against 1.10 per cent in that from the check plat. Again, the straw from the lime and manure plat analyzed 1.02 per cent K₂O as compared with a K₂O occurrence of 1.21 per cent in the straw harvested from the plat which received manure alone. The entire crop from the untreated plat removed 16.1 pounds of K₂O per acre, while that from the lime plat removed 15.6 pounds. The plat which received 6 tons of manure without lime yielded K₂O at the rate of 37.6 pounds per acre, as against 34.9 pounds from the plat which received both lime and manure.

In summarizing the results from the Rhode Island Agricultural Experiment Station, Wheeler (34) concluded that a study of the soils of that state does not justify the assumption that lime is to be considered as an extensive liberant of soil potash.

Briggs and Breazeale (4) found that solutions of Ca(OH)₂ were inactive upon the potassium content of soil and also upon that of both pegmatite and orthoclase; while the solubility of the potash content of orthoclase was also depressed by CaSO₄. Calcium was likewise found to induce no acceleration in the absorption of potassium by wheat seedlings, when the seedlings were grown in contact with soil and orthoclase. Had there been present an abundance of potash resultant from liberation effected by treatment, the reverse would have been expected, for LeClerc and Breazeale (15) demonstrated that there existed a marked tendency on the part of the seedlings to take up particularly large proportions of potash at this stage of growth.

As a result of his pot and laboratory studies with a poor soil supplemented by additions of muscovite, biotite, orthoclase and microline, Plummer (27),

concluded that $CaCO_3$ treatment and calcium bicarbonate solutions do not, *per se*, increase the amount of potash available to the plant, as measured by either the plant or by N/5 acid. Lipman and Gericke (16) studied potash liberation induced by $CaCO_3$ and $CaSO_4$ from two soils, an Oakley blow sand and a Berkley adobe and also a greenhouse modification of the adobe soil, the latter having been made by mixtures of the original adobe soil and barnyard manure. Treatments were mixed throughout and moist contact permitted. After periods of approximately 1, 4, and $9\frac{1}{2}$ months for the Oakley soil, and $1\frac{1}{2}$, 4, and 10 months for the adobe soil, with somewhat comparable periods for its manure modification, 1:2 proportions of soil and distilled water were allowed to digest for 6 days prior to filtration through Pasteur-Chamberland pressure filters. The water solutions thus obtained showed no increase in potash from either the $CaCO_3$ or $CaSO_4$ treatment in the case of the blow sand. Some increase was obtained in the filtrations from the adobe soil, as a result of both $CaCO_3$ and $CaSO_4$ additions; and the same was true of its manure-mixture modification. The longer periods of contact induced no co-incidental increase in water-soluble potash. The greenhouse modification gave a greater potash recovery than did the unmodified soil, though it is not evident what part of the increase is attributable directly to the potash content of the manure added. Working with 500-gm. portions of soil and maintaining contact with 60 per cent moisture-holding capacity for 19 weeks at 30°C., Ames and Boltz (1) secured data indicating the liberation of potassium from added or engendered $CaSO_4$, but not from additions of $CaCO_3$. The analyses were made upon extractions of distilled water in the proportion of 400 gm. of soil to 2500 cc. of water. In the case of the silt loam soil, $CaCO_3$ treatment depressed the extractable potassium, but the addition of dried blood, with and without $CaCO_3$, effected an enhanced solubility of native potassium.

FIELD STUDIES

The effect of lime as a liberative agency in increasing the availability of soil potash has been studied but little under practical field conditions, with supplementary laboratory investigations. The plats of the Pennsylvania station offer a splendid opportunity for such a study, if it is assumed that adequate accuracy of sampling can be attained. Such a study was made by the writer (20) in 1911. The results were reported as a part of a general lime study and would not be found easily, if at all, in a search for data on potash experiments. Then too, the potash results were but incidental in the general study and the discussion offered was therefore limited. In addition, the annual reports attain only a limited circulation. The results are therefore given *in toto* (table 1).

In this work, four similarly treated plats of the same numeral designation one from each tier, were sampled. Composite samples were made from ten borings taken from each of the four $\frac{1}{4}$-acre plats, at each of three depths. The total residual potash found in the upper 21 inches was taken as a measure of the activities of the several forms of lime in activating the outgo of potash.

Discussion of the data of table 1

In comparing the manure plats no. 16 with the lime and manure plats no. 22, we find that the two lower depths of the former give an average of 69,236 pounds as against 68,899 pounds for the latter, or practically identical results. However, the upper 7 inches of the lime and manure plats no. 22 had approximately 9000 pounds less of K$_2$O than had the corresponding zone of the plats receiving manure alone. This comparison would seem to indicate a considerable potash replacement in the surface zone, without any fixation of the liberated surface soil potash, as it leached down through the underlying zones. However, had potash been liberated by the additions of lime, it would seem

TABLE 1

The influence of 30 years of liming upon the residual potassium of the upper three 7-inch zones of Hagerstown silty clay loam of the Pennsylvania Station General Fertilizer Experiment plats—equal amounts used from each of the four tiers in making composite samples

SOIL ZONES SAMPLED	K$_2$O FOUND, POUNDS PER ACRE					
	Plats 16	Plats 22	Plats 23	Plats 24	Plats 33	Plats 34
	6 tons of manure biennially	2 tons CaO quadren- nially and 6 tons of manure biennially	2 tons CaO quadren- nially	Checks	320 pounds of gypsum biennially	2 tons ground lime- stone bien- nially
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
0–7 inches...............	75,544	66,574	66.523	68,532	72,440	69,154
7–14 inches...............	72,645	70,076	68,714	68,673	67,312	65,653
14–21 inches...............	65,827	67,721	66,523	76,136	66,410	57,705
0–21 inches...............	214,016	204,371	201,760	213,341	206,162	192,512

* 2,047,500 pounds per acre 7 inches is used for calculations to pounds per acre.

that the clay subsoil would have undoubtedly functioned as an absorbent for downward moving potassium salts, as will be shown was the case with the similar Cumberland loam soil of the lysimeters.

In a comparison between the analyses of the limed plats no. 23, with the limed and manured plats no. 22, we find the surface zones to be practically identical in composition, while the lower zones average 67,618 pounds for plats no. 23, as against an average of 68,999 pounds for the corresponding zones of plats no. 22. Considering the mass of soil as represented by the soil-weight used in the calculations, and without attributing a part of the difference to potash absorbed from that supplied by the 90 tons of manure, the results are reasonably concordant. Where these two limed plats, no. 22 and 23, are compared with their corresponding checks, no. 16 and 24, some displacement of potassium is indicated. It should be noted, however, that while the upper two zones of the untreated plats no. 24 are practically identical, the lower zone

jumps to a content of 76,136 pounds. If it were assumed that the lowest zone of plats no. 24 was not truly representative and the average of the two upper zones were instead assigned to it, then in a comparison with plats no. 22 and 23 this hypothetical summation would indicate a loss of only 1437 pounds of K_2O in the case of the limed and manured plats no. 22 and but 4040 pounds for plats no. 23 which received lime alone. However, the total potash in the upper 21 inches of the control plats no. 16, which received manure alone, amounts to 214,016 pounds, as against 213,341 pounds for the check plats no. 24. Again, the substitution of the average amount of the upper two zones for the determined amount found in the lowest zone would give a total of 205,808 pounds for the check plats, as against 206,162 for the gypsum plats. Thus the $CaSO_4$ treatments amounting to 4,800 pounds over a 30-year period would have registered no potash liberation, if the average composition figure were used to replace that determined. It seems hardly probable that the sampling of the lowest zone of the composite of plats no. 24 is truly representative; however, the two comparisons, that of the total from the composite of the check plats no. 24 against that of the total of the composites of the control plats no. 16 and that of the check plats no. 24 against the gypsum-treated plats no. 33, would militate against the adoption of the total which would obtain, were the apparent error in the sampling of the lowest zone to be presumed as being rectified by the substitution of the value found by the process of averaging the figures obtained in the analyses of the two upper zones.

In further comparisons, the composite of the ground-limestone plats no. 34 shows a deficiency of potash amounting to 20,829 pounds for the entire 21 inches, as compared to the residual potash found in the composite of the check plats no. 24. This difference is manifestly of greater magnitude than would be chemically possible, in view of the determinations of the total residual lime and residual calcium carbonate at the same depths. Here, again, the third zone appears as abnormal, and since the apparent abnormality of the third zone sampling of plats no. 24 tends upward and that of plats no. 34 tends downward, the differential error is accentuated. It would, of course, be contrary to all chemical conceptions to assume that the amount of potash liberated by the ground limestone would be equal to that set free by the burnt lime under the imposed conditions of repetition over such a long period.

Considering the comparisons from another angle, it is very interesting to note that had the analyses been confined to the samplings from the two upper zones, and in particular had the manure control plats not been included, the conclusions would have been definitely and materially different. No potash liberation would have been indicated, 137,205 pounds having been found in the upper 14 inches of the composited check plats against 139,752 pounds for the gypsum plats. On the other hand, the comparison of 137,205 pounds for the check plats against 136,650 pounds, 135,237 pounds, and 134,807 pounds, would give a loss of potash amounting, respectively, to 555 pounds for the lime and manure plats, 1968 pounds for the burnt-lime plats and 2308 pounds for

the ground-limestone plats. Looking at the data from either viewpoint, that of the upper 14 inches, or that of the aggregate of the three 7-inch zones, but with reservation as to the commensurateness of sampling and analysis, it would seem reasonable to assume that the burnt-lime and ground-limestone treatments have effectuated some, though small, release and leaching of potash. Remembering the much lighter application of CaSO$_4$ and considering the mass of the soil sampled, the action of the total of 4800 pounds of CaSO$_4$ may be said to have been indefinitely established.

In discussing these results, in this connection, it is thought well to point out the limitations incident to representative sampling, even when there is exerted the most extreme care and the further limitation of interpretation of analytical results into positive terms on the acre depth basis. The accuracy and dependability of supposedly representative sampling are in no wise commensurate with the analytical procedure carried out in the laboratory. The results embodied in table 1 represent the true potassium content of the samples obtained; but the uncertainty as to their representative character necessitates reservations in the interpretation and acceptance of the findings and indications derived therefrom. Such difficulties and limitations possibly serve to emphasize the value and greater dependability of data secured through the medium of carefully controlled lysimeter experiments, where careful mixing of the soil mass assures a reasonably uniform composition of the soil used to fill the tanks.

Previous lysimeter investigations

As compared with laboratory extractions and percolating studies, there is a dearth of lysimeter investigations upon the subject of potash liberation and outgo induced by lime treatments. Such studies have been considered by some (Lipman (16), Tressler (32)) as being more indirect than direct. To the writer, it would appear that the actual static equilibria would be much more nearly indicated by the leachings obtained from exposure to actual precipitation, than by extractions under the accentuated conditions imposed during agitation with a surplus of solvent, after an exaggerated period of contact of treatment and soil, such as would not be encountered if the factors of solubility, outgo and absorptive tendencies were given free play. This would be particularly true in the case of the batteries where no subsoil was used.

Lyon and Bizzell (17, 18) have recently reported the results secured during the initial 5-year period in their lysimeter studies upon a Dunkirk clay loam. The analysis of leachings gave no indication of any liberation of potassium to the free soil water as a result of one application of burnt lime at the rate of 3000 pounds per acre. However, since the applications of potassium sulfate likewise failed to show any enrichment in the potassium content of leachings, it is recognized that the underlying 3 feet of soil might have effected the absorption of some fraction of the total of potassium salts which might have

passed from the surface soil. Although the average crop analyses show that the plants utilized about twice as much potassium as was leached under fallow conditions, nevertheless the outgo of potassium in leachings is apparently independent of the crop growth. This would suggest the possibility that the crops derived their stores of potassium from the potassium content of film water, rather than from that contained in the free water. The averages from annual analyses of corn, oats, wheat and grass for 4 years show a practically identical potassium content in the crops from the limed and unlimed tanks.

In their comprehensive memoir, Lyon and Bizzell (18) review the several aspects resulting from the data derived from lysimeter investigations reported. The results reported from the Florida, Texas, Hawaiian, Rothamsted, Bromberg, Göttingen, Jönköping and Cawnpore stations also are reviewed in some detail. From some of the above cited data and other publications, it might be assumed, and indeed it is generally held, that the loss of potassium from the soil through leaching is to be considered as negligible. Soil type, composition, age, method of cropping, periodicity and magnitude of rainfall, topography, range of seasonal temperature and other factors must have important bearing upon the extent of potassium outgo, which may truly have very wide range. Clarke (7) in offering the results of analyses of 203 samples of drainage water shows that, while more often occurring in relatively small amounts, potassium may be found as high as 160 parts per million. Greaves and Hirst (14) record the analyses of the water from 67 streams, 4 drains and 3 wells, giving amounts varying from a minimum of 0.79 parts per million to 25.3 parts per million. The maximum figure offered by them is greater than that secured in any annual period in the results offered in this paper. This is true even in the case of the 100-ton lime tanks.

LIME AND MAGNESIA LYSIMETER STUDIES AT THE TENNESSEE STATION

Storer (31) attributes to gypsum the function of liberation of soil potash, followed by its dissemination throughout a more extensive area, thus increasing the feeding zone of growing plants in search of nutrient potash. The lysimeter studies offered in this treatise are so planned that by the use of soil alone and soil with subsoil, any such migration following release by treatment may be recorded and some measure of the absorptive tendencies of the soil and subsoil obtained, thus throwing some light upon the phase of the problem pointed out by Storer.

In previously reported studies (21) based upon the use of Cumberland loam soil in field cylinders, different forms of lime were used in 2-ton and 8-ton per acre applications. As a result of those and other studies, further experiments were planned and begun in July, 1914, using lysimeters in which the minimum applications of burnt lime were at the rate of 8 tons, equivalent to the maximum amount used in the cylinder investigations. The lysimeter equipment has been previously described and illustrated (21) and is also shown in this contribution

(plate 1). Treatments equivalent to 32 tons and 100 tons per acre were also included in the later lysimeter experiments. In amounts chemically equivalent to these three amounts of CaO, treatments of MgO, precipitated $CaCO_3$, precipitated $MgCO_3$, 100-mesh limestone, 100-mesh dolomite, and 100-mesh magnesite were also included in the scheme of experimentation. Thirty-two-ton applications of wollastonite and of serpentine of 100-mesh fineness were also used in the deep tanks. Thus, in the surface-soil group, there were included, three CaO treatments, one each at the rates of 8, 32 and 100 tons per acre 2,000,000 pounds of soil. In like manner, three tanks of each of the other six, chemically equivalent oxide and carbonate materials were utilized. In this way, three surface-soil batteries of 7 tanks each, at the three rates, were incorporated in the experiment. These 21 surface-soil tanks were then duplicated exactly, save for the fact that the surface soil of the second series of 21 tanks rested upon 1 foot of well tamped, untreated clay subsoil. In addition there were also installed one blank surface-soil-only tank and one surface-and-subsoil tank.

The periodical leachings from the several tanks have been collected over a period of 5 years and a definite aliquot of each sample preserved in acid solution. Each annual composite thus obtained has been subjected to "complete" chemical analysis. This series of the 46 annual composites, for each of 5 years affords an excellent opportunity to gauge any enhanced outgo of potassium as the result of applications of nine different calcic and magnesic compounds in amounts up to the equivalence of 200,000 pounds of CaO per acre 2,000,000 pounds of soil.

In addition to the foregoing system of 46 tanks devoted to the study of the Cumberland loam, another battery of 22 tanks containing Cherokee sandy loam, surface soil only, has been under experimentation for 2 years. The leachings from this second set have been preserved and analyzed in the same manner as were those of the system of 46 tanks. While devoted primarily to the study of the outgo of sulfur from treatments of both native and applied sulfur, the second system affords an additional opportunity for study of the liberation of native soil potassium, as indicated by leachings. The treatments of this set will be described in prefacing the report of the results secured during the initial 2-year period and recorded in the latter part of this contribution.

In the scheme of experimentation with the two soils we have, therefore, a study embracing 11 calcic and magnesic treatments at several rates, from 9 additions, namely, CaO, MgO, $CaCO_3$, $MgCO_3$, limestone, dolomite, magnesite, wollastonite and serpentine; and 2 engendered compounds $CaSO_4$ and $MgSO_4$, where sulfur was added as $FeSO_4$, iron pyrites or sulfur together with lime or magnesia. Indeed, at certain periods, the formation of calcium and magnesium nitrates was of such magnitude as to be actually equivalent to treatments of those compounds.

The potassium content of the Cumberland loam and the Cherokee sandy loam

The potassium content *in toto*, and the relative solubilities of the aggregate of the potassic complexes are shown in table 2. The total potassium content was obtained by the J. Lawrence Smith disintegration procedure, described by Crookes (8). The N/5 acid digestion was at room temperature, with frequent agitations over a period of 5 hours. The HCl 1.115 digestion was carried out for 10 hours at the temperature of boiling tap water. The two samples of Cumberland loam were secured from approximately the same spot, but at different times. The two samples show close concordance in their amounts of acid-soluble potassium and total potassium. The clay subsoil used in tanks 22–44 and that used in tank 45 were from beneath the same soil,

TABLE 2

Acid-soluble and total potassium content of Cumberland loam and Cherokee sandy loam (moisture-free basis)

LABORA-TORY NUMBER	SOIL	USED IN TANKS NUMBER	POTASSIUM CONTENT					
			N/5 HNOₐ digestion 220-gm. charge		HCl 1.115 digestion 10-gm. charge		J. L. Smith fusion 1-gm. charge	
			Per cent	Pounds per 2,000,000 pounds of soil	Per cent	Pounds per 2,000,000 pounds of soil	Per cent	Pounds per 2,000,000 pounds of soil
3343	Cumberland loam......	1–44	0.0097	194	0.244	4,880	1.004	20,080
4693	Cumberland loam......	45–46	0.0103	206	0.248	4,960	1.007	20,140
3224	Subsoil...............	22–44			0.618	12,360	0.898	17,960
4694	Subsoil...............	45	0.0110	220	1.100	22,000	1.445	28,900
4893	Cherokee sandy loam...	50–71	0.0064	128	0.243	4,860	0.753	15,060

but both could not be secured from exactly the same spot because of building operations between the periods of sampling. Some difference in potassium content of the two subsoils is indicated by the analyses. As would be expected, the Cherokee sandy loam reflects its type in the potassium content determined by the analyses.

The 46-tank lime-magnesia system data

Tables 3 to 14 and figures 1 to 15

The potassium determinations upon the several annual composites from the original 46 tanks are given in tables 3 to 14, inclusive. The results were obtained by the use of the colorimetric method of Cameron and Failyer (6), as given by Shreiner and Failyer (29). Each annual outgo of potassium from both surface-soil and surface-soil-plus-subsoil tanks will be considered in order of sequence.

Initial annual period (tables 3 and 4). The volumes of leachings were fairly concordant, when the average of the three 7-tank surface-soil batteries are

TABLE 3

Amounts of potassium leached from Cumberland loam, surface soil only, in field lysimeters— July, 1914, to July, 1915

Treatments of CaO, MgO, CaCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons, and 100 tons per acre 2,000,000 pounds of soil

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with soil	Rate per 2,000,000 pounds of soil				
		tons				
1	CaO	8	69.7	4.98	0.3472	15.3
2	MgO	∞8	69.2	3.19	0.2205	9.7
3	CaCO₃	∞8	74.4	7.47	0.5557	24.5
4	MgCO₃	∞8	77.3	4.58	0.3542	15.6
5	Limestone	∞8	82.0	3.98	0.3267	14.5
6	Dolomite	∞8	67.2	2.44	0.1639	7.2
7	Magnesite	∞8	74.0	1.84	0.1363	6.0
	Average	∞8	73.4	4.07	0.3006	13.3
8	CaO	32	79.8	4.28	0.3417	15.1
9	MgO	∞32	74.8	1.73	0.1292	5.7
10	CaCO₃	∞32	81.5	3.98	0.3247	14.3
11	MgCO₃	∞32	57.0	2.31	0.1315	5.8
12	Limestone	∞32	81.6	2.24	0.1828	8.1
13	Dolomite	∞32	78.2	1.73	0.1350	6.0
14	Magnesite	∞32	81.7	2.09	0.1708	7.5
	Average	∞32	76.4	2.62	0.2022	8.9
15	CaO	100	79.2	3.98	0.3156	13.9
16	MgO	∞100	72.9	2.59	0.1889	8.3
17	CaCO₃	∞100	68.5	2.37	0.1625	7.2
18	MgCO₃	∞100	61.1	2.59	0.1582	7.0
19	Limestone	∞100	82.4	2.37	0.1955	8.6
20	Dolomite	∞100	61.0	5.26	0.3208	14.1
21	Magnesite	∞100	71.8	1.73	0.1241	5.5
	Average	∞100	71.0	2.98	0.2094	9.2
Average of all treated tanks			73.6	3.22	0.2371	10.5

compared. The potassium concentrations of the leachings from the 8-ton tanks average somewhat higher than do those from the heavier treatments of 32 and 100 tons. The CaO treatments appear to have exerted a greater tendency toward potassium outgo than did the corresponding MgO treatments.
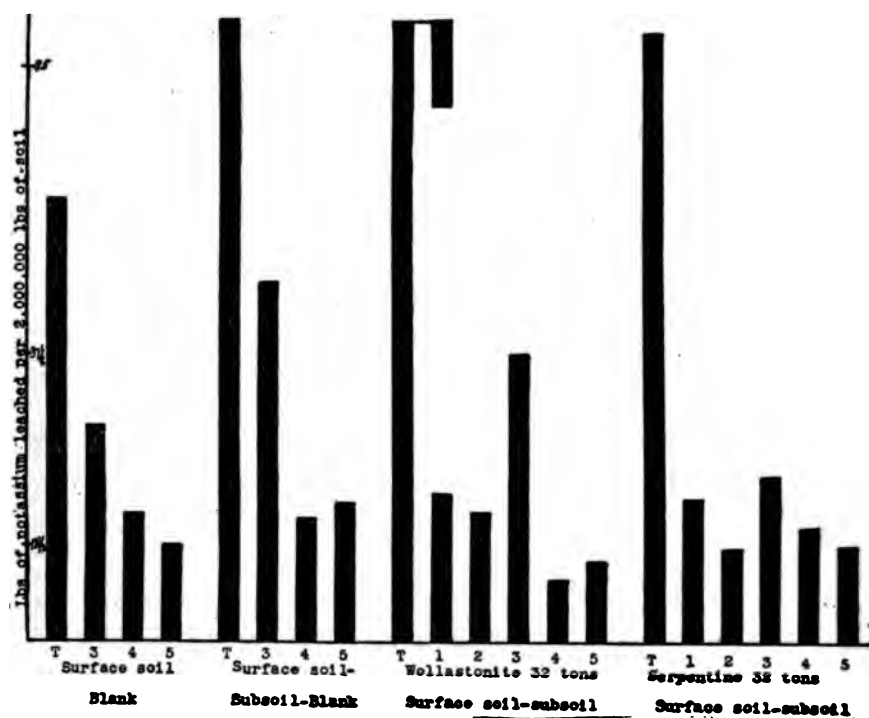
FIG. 1. No-Treatment, Wollastonite and Serpentine Tanks, Cumberland Loam
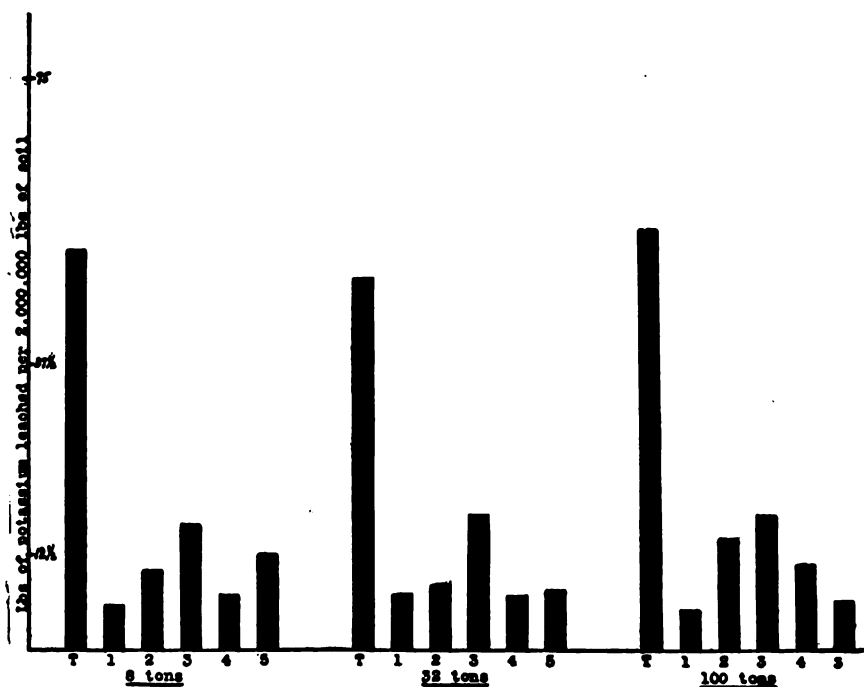T = total; numbers refer to annual periods
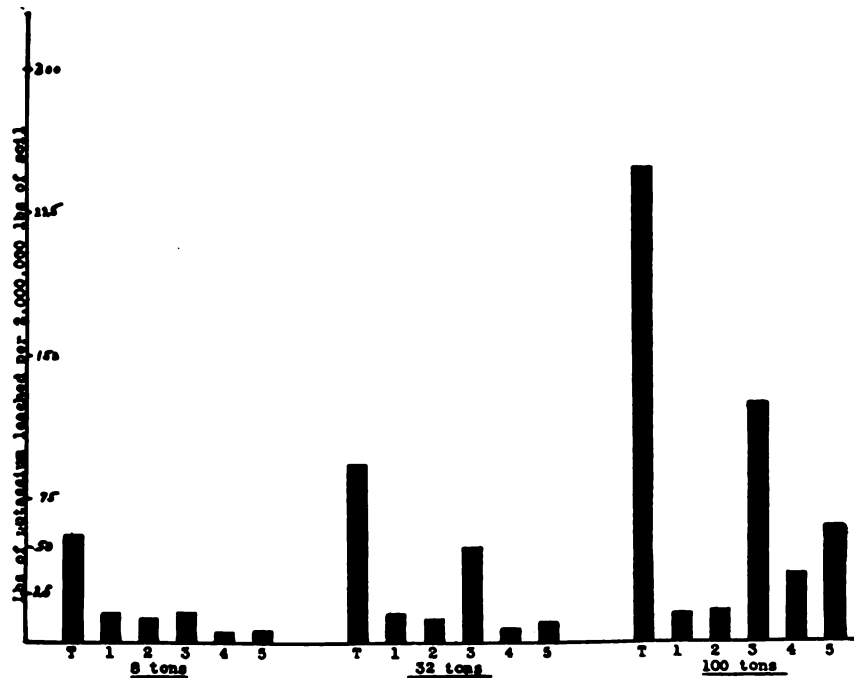
FIG. 2. Burnt Lime Treatments, Surface-Soil-Only Tanks, Cumberland Loam
T = total; numbers refer to annual periods

TABLE 4

Amounts of potassium leached from Cumberland loam, surface soil and subsoil, in field lysimeters—
July, 1914, to July, 1915

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and 100 tons per acre 2,000,000 pounds of soil—also wollastonite and serpentine at the rates of 32 tons

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with surface soil	Rate per 2,000,000 pounds of soil				
		tons				
22	CaO..........................	8	73.2	5.48	0.4008	17.7
23	MgO..........................	○8	66.4	5.38	0.3569	15.8
24	CaCO₃........................	○8	76.6	3.98	0.3050	13.5
25	MgCO₃........................	○8	55.4	5.98	0.3309	14.6
26	Limestone....................	○8	63.3	4.98	0.3153	13.9
27	Dolomite.....................	○8	67.0	5.98	0.4006	17.7
28	Magnesite....................	○8	75.4	4.28	0.3229	14.2
	Average....................	○8	68.2	5.15	0.3475	15.3
29	CaO..........................	32	73.2	4.58	0.3352	14.8
30	MgO..........................	○32	62.7	5.98	0.3749	16.5
31	CaCO₃........................	○32	72.3	5.07	0.4825	21.3
32	MgCO₃........................	○32	84.6	5.48	0.4636	20.4
33	Limestone....................	○32	71.1	4.98	0.3542	15.6
34	Dolomite.....................	○32	75.2	4.28	0.3222	14.2
35	Magnesite....................	○32	84.2	3.98	0.3354	14.8
	Average....................	○32	74.8	4.91	0.3811	16.8
36	CaO..........................	100	87.5	4.28	0.3747	16.5
37	MgO..........................	○100	90.2	4.28	0.3862	17.0
38	CaCO₃........................	○100	54.7	4.98	0.2724	12.0
39	MgCO₃........................	○100	55.5	1.33	0.0737	3.3
40	Limestone....................	○100	91.7	7.47	0.6848	30.2
41	Dolomite.....................	○100	80.1	5.48	0.4387	19.3
42	Magnesite....................	○100	62.4	3.98	0.2488	11.0
	Average....................	○100	74.6	4.54	0.3542	15.6
43	Wollastonite.................	32	80.1	5.48	0.4390	19.4
44	Serpentine...................	32	85.3	3.98	0.4245	18.7
Average of treated tanks, except no. 43 and 44..			72.5	4.87	0.3609	15.9
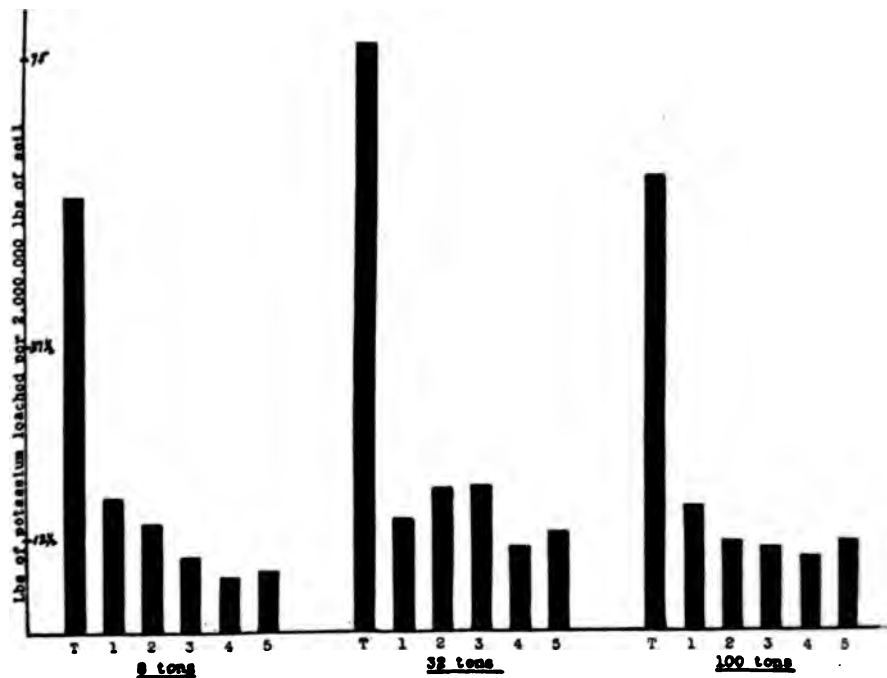
FIG. 3. BURNT LIME TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS, CUMBERLAND LOAM
T = total; numbers refer to annual periods
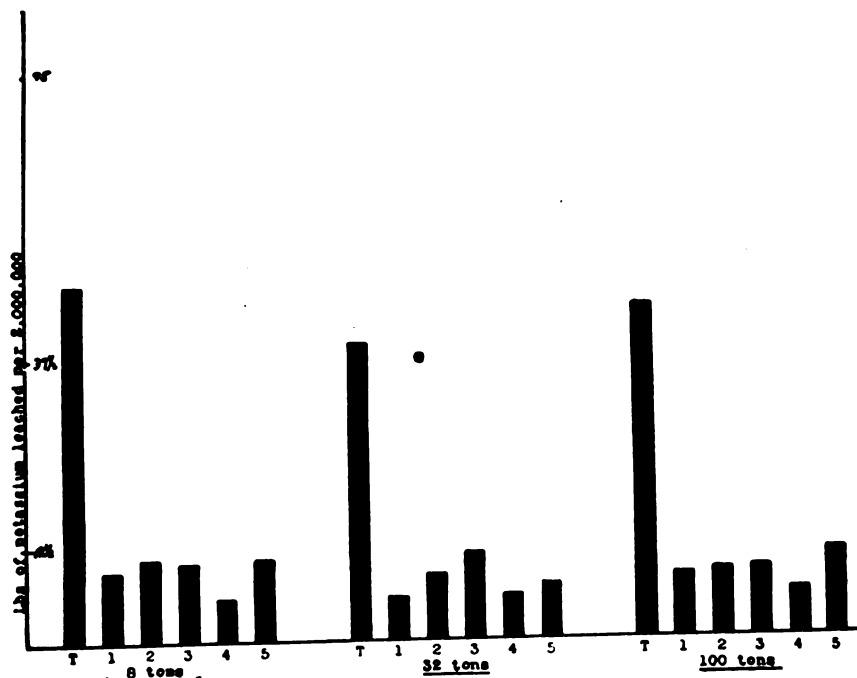
FIG. 4. MgO TREATMENTS, SURFACE-SOIL-ONLY TANKS, CUMBERLAND LOAM
T = total; numbers refer to annual periods

However, the extent of potassium leaching was not great in any instance, the maximum average being but 13.3 pounds per 2,000,000 pounds of soil. While the blank was not included during the initial two years, the first annual analysis of leachings from the untreated surface soil showed an outgo of 28.2 pounds during the third year, as against 46.7 pounds from the subsoil tank; however, the average of the 21 treated surface-soil tanks gave a volume leached of 167.6 litres for the third year, as compared to a corresponding average of 73.6 litres for the initial year. These comparisons would indicate no potash liberation, if not an actual depressed outgo during the initial year, as a result of surface-soil treatment without underlying subsoil.

A comparison between the 21 surface-soil tanks and the correspondingly treated tanks containing subsoil shows a slightly less average volume leaching, but a heavier average outgo of potassium. As a whole, the subsoil tanks gave an average potassium outgo of 15.9 pounds against an average of but 10.5 pounds for the surface soil. The most outstanding exception to this relationship is that of tank 39, which suffered a loss of but 3.3 pounds of potassium. However, because of the ameliorating effects induced by screening and placing, the results of the first year would hardly be considered as representing the normal relationship between the soils of the deep and shallow tanks. The data indicate that, during the initial year, the effect of screening and placing the subsoil was to accelerate its tendency to yield potassium to solution, rather than to enhance its absorptive properties.

Second annual period (tables 5 and 6). During the second year of exposure, the potassium leachings from the surface-soil and subsoil tanks were more nearly equivalent. The average outgo from the seven surface-soil tanks, each receiving 8-ton CaO equivalence, was 10.3 pounds as compared with 12.9 pounds for the corresponding deep cans; while the 32-ton surface tanks yielded 9.9 pounds as an average against 14.4 pounds for the corresponding subsoil tanks. On the other hand, the 100-ton treatments gave practically identical averages, namely, 13.2 pounds and 13.1 pounds, respectively, for the corresponding surface-soil-subsoil tanks. The second annual period gave 16.9 pounds for the surface-soil blank as against 16.1 pounds for the subsoil blank. The difference between the results induced by the CaO and MgO treatments was not nearly so marked nor extensive during the second year, especially in the case of the subsoil tanks.

All of the tanks have been analyzed at several periods for occurrences of residual $CaCO_3$. The results showing these residual occurrences have been offered by the writer (21). These data demonstrate that during most of the first year and thereafter, the 8-ton CaO treatments may be considered as being identical to treatments of $CaCO_3$, all of the oxide having reverted to the carbonate. The MgO and $MgCO_3$ 8-ton treatments, however, do not maintain the same parallel in carbonation. These results are to be later offered in detail. Suffice it to say that the applied $MgCO_3$ had undergone practically complete disintegration, resulting in liberation of CO_2 and fixation of magnesium; while
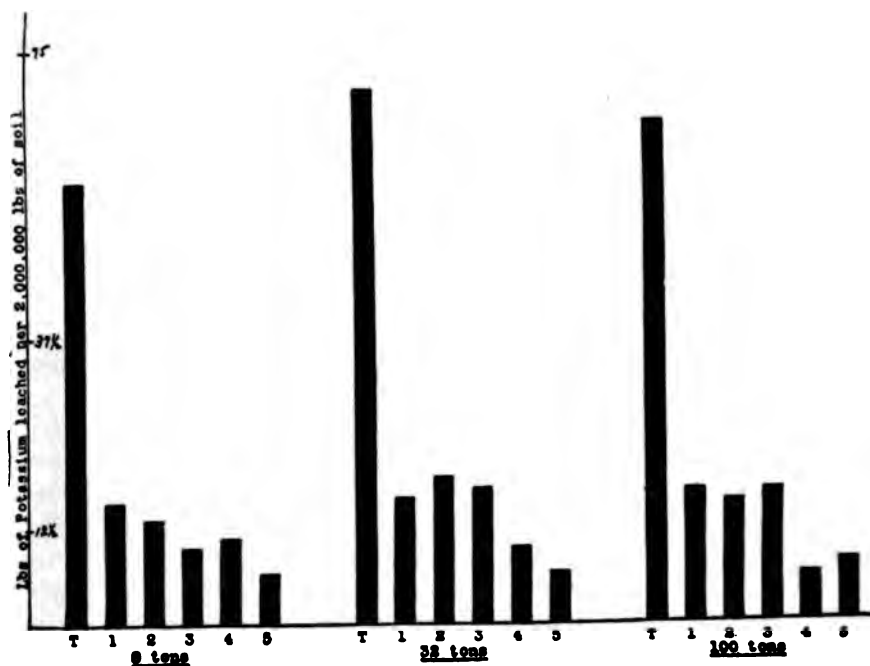
FIG. 5. MgO TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS, CUMBERLAND LOAM
T = total; numbers refer to annual periods

FIG. 6. PRECIPITATED CaCO₃ TREATMENTS, SURFACE-SOIL-ONLY TANKS, CUMBERLAND
LOAM
T = total; numbers refer to annual periods

TABLE 5

ounts of potassium leached from Cumberland loam, surface soil only, in field lysimeters—July, 1915, to July, 1916

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and -mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and tons per acre, 2,000,000 pounds of soil

NK BER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with soil	Rate per 2,000,000 pounds of soil				
		tons				
1	CaO......................	8	136.8	1.99	0.2725	12.0
2	MgO......................	∞8	116.3	2.21	0.2567	11.3
3	CaCO₃....................	∞8	116.7	1.48	0.1716	7.6
4	MgCO₃....................	∞8	143.7	2.09	0.3005	13.3
5	Limestone.................	∞8	129.2	1.59	0.2058	9.1
6	Dolomite..................	∞8	107.7	1.81	0.1948	8.6
7	Magnesite.................	∞8	130.0	1.81	0.2352	10.4
	Average.................	∞8	125.8	1.85	0.2339	10.3
8	CaO......................	32	113.9	2.32	0.2638	11.6
9	MgO......................	∞32	108.1	1.81	0.1956	8.6
0	CaCO₃....................	∞32	118.5	3.02	0.3580	15.8
1	MgCO₃....................	∞32	98.9	1.48	0.1461	6.4
2	Limestone.................	∞32	124.7	1.48	0.1842	8.1
3	Dolomite..................	∞32	127.0	1.81	0.2298	10.1
4	Magnesite.................	∞32	137.7	1.43	0.1965	8.7
	Average.................	∞32	118.4	1.91	0.2249	9.9
5	CaO......................	100	107.4	3.32	0.3566	15.7
6	MgO......................	∞100	96.5	2.12	0.2019	8.9
7	CaCO₃....................	∞100	104.1	3.62	0.3767	16.6
8	MgCO₃....................	∞100	96.9	3.32	0.3217	14.2
9	Limestone.................	∞100	121.3	2.34	0.2839	12.5
0 .	Dolomite..................	∞100	112.0	2.12	0.2342	10.3
1	Magnesite.................	∞100	132.5	2.49	0.3299	14.5
	Average.................	∞100	110.1	2.76	0.3007	13.2
	verage of all treated tanks.................		118.1	2.17	0.2532	11.1

y carbonation of MgO was followed by the disintegration of the resultant ·bonate. Therefore, during most of the first year and thereafter, the 8-ton ;O and MgCO₃ treatments are in effect treatments of freshly formed magium silicates, of simple or complex nature. The 32-ton and 100-ton MgO 1 MgCO₃ treatments, however, are in excess of the amounts which the soil s capable of disintegrating. In these cases the treatments represent MgO, bably some magnesium hydrate, basic carbonate and silicate, the latter
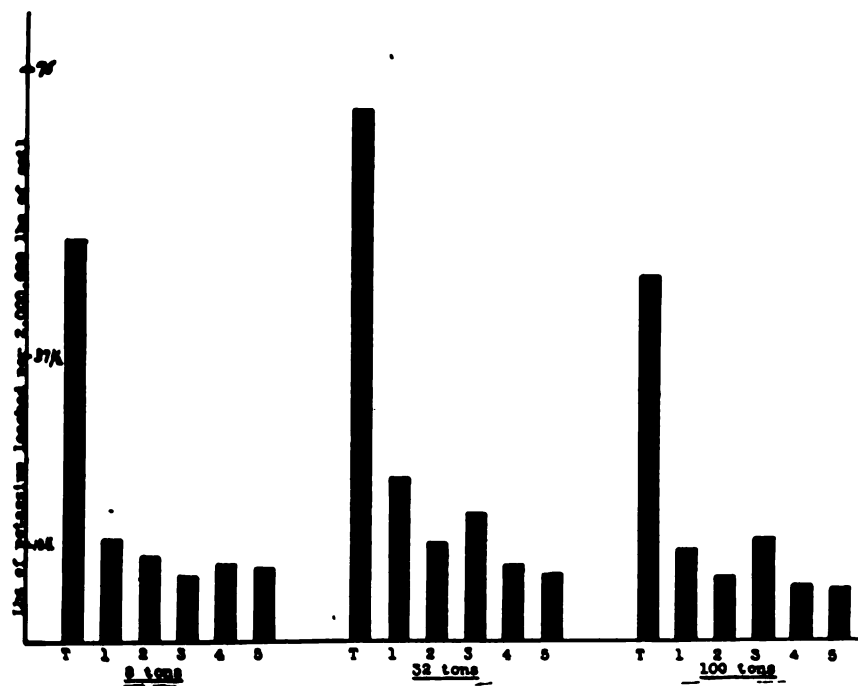
FIG. 7. PRECIPITATED CaCO₃ TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods
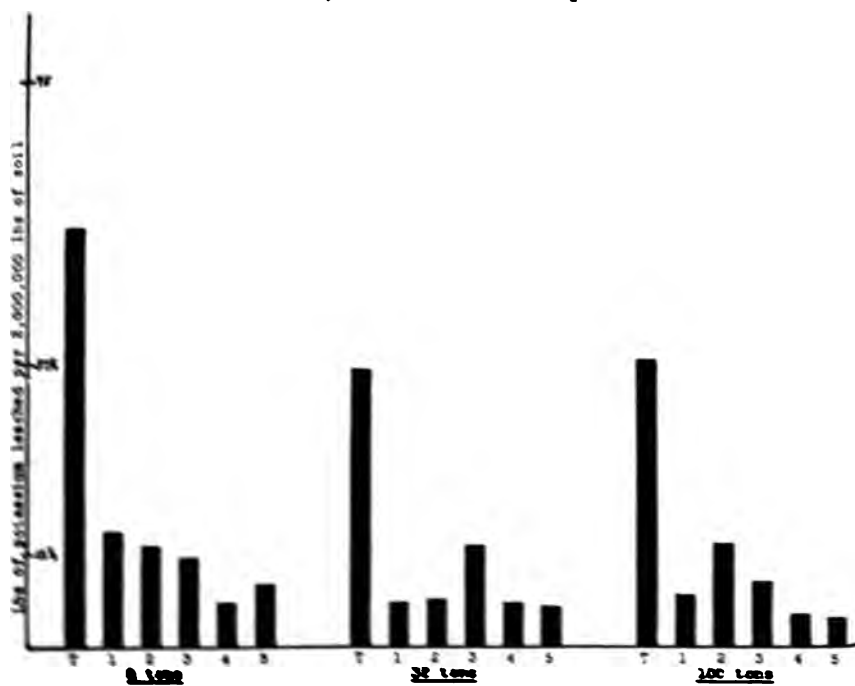
FIG. 8. PRECIPITATED MgCO₃ TREATMENTS, SURFACE-SOIL-ONLY TANKS, CUMBERLAND
LOAM
T = total; numbers refer to annual periods

TABLE 6

Amounts of potassium leached from Cumberland loam, surface soil and subsoil, in field lysimeters—July, 1915, to July, 1916

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and -mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and tons per acre 2,000,000 pounds of soil; also wollastonite and serpentine at the rate of :ons

NK BER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with surface soil	Rate per 2,000,000 pounds of soil				
		tons				
2	CaO	8	121.8	2.65	0.3224	14.2
3	MgO	∞8	117.5	2.65	0.3111	13.7
4	CaCO₃	∞8	120.4	2.09	0.2518	11.1
5	MgCO₃	∞8	114.0	2.65	0.3019	13.3
6	Limestone	∞8	114.0	2.34	0.2668	10.9
7	Dolomite	∞8	119.5	2.65	0.3164	14.0
8	Magnesite	∞8	128.3	2.34	0.3002	13.2
	Average	∞8	119.3	2.62	0.2958	12.9
9	CaO	32	108.9	3.98	0.4338	19.1
0	MgO	∞32	108.0	3.98	0.4303	19.0
1	CaCO₃	∞32	110.4	2.65	0.2923	12.9
2	MgCO₃	∞32	109.6	2.84	0.3111	13.7
3	Limestone	∞32	109.5	3.07	0.3363	14.8
4	Dolomite	∞32	116.7	2.34	0.2732	12.0
5	Magnesite	∞32	113.7	1.81	0.2058	9.1
	Average	∞32	111.0	2.65	0.3261	14.4
6	CaO	100	121.5	2.21	0.2683	11.8
7	MgO	∞100	106.5	3.32	0.3536	15.6
8	CaCO₃	∞100	104.3	1.81	0.1887	8.3
9	MgCO₃	∞100	93.3	3.02	0.2819	12.4
0	Limestone	∞100	117.7	2.49	0.2931	12.9
1	Dolomite	∞100	126.7	2.49	0.3155	13.9
2	Magnesite	∞100	114.5	3.32	0.3801	16.8
	Average	∞100	112.1	2.66	0.2973	13.1
3	Wollastonite	32	135.2	2.84	0.3839	16.9
4	Serpentine	32	136.5	1.99	0.2719	12.0
Average of treated tanks, except no. 43 and 44			114.1	2.64	0.3064	13.5
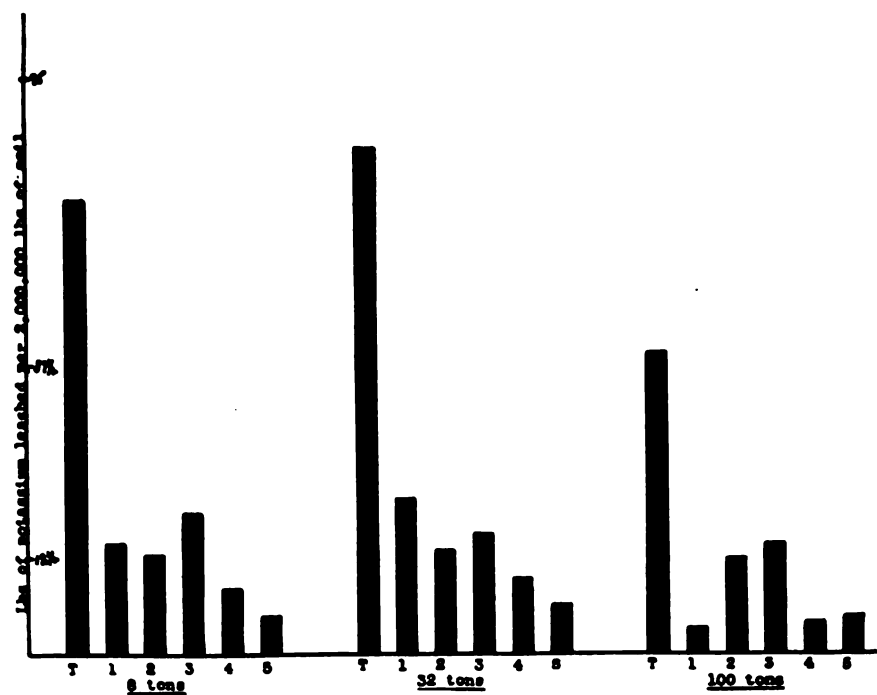
FIG. 9. PRECIPITATED MgCO₃ TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods
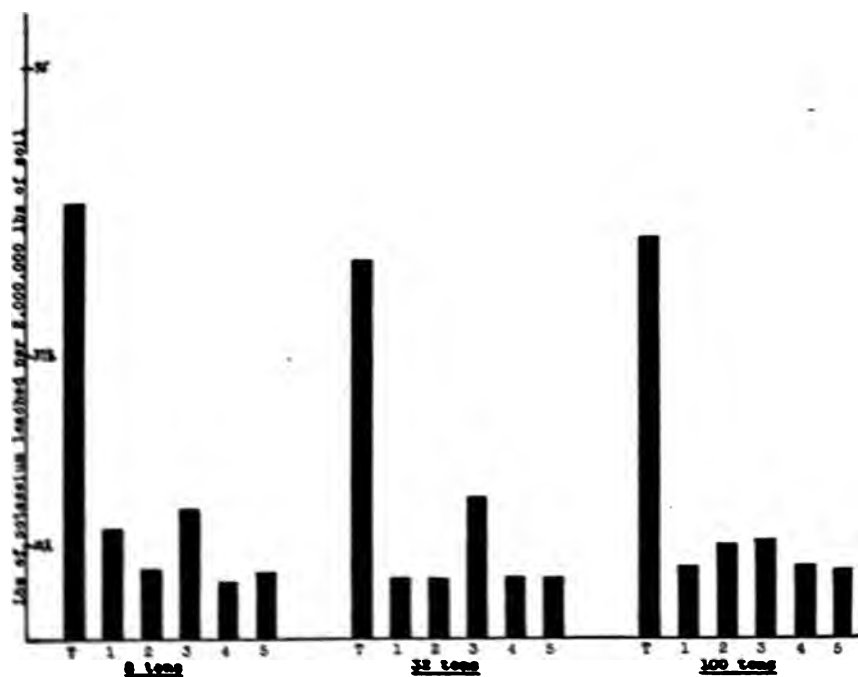
FIG. 10. 100-MESH LIMESTONE TREATMENT, SURFACE-SOIL-ONLY TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods

being in .excess of equivalence to the amount of calcium silicate formed by applications of lime in amounts chemically equivalent to the applied magnesia and magnesium carbonate.

Again in the second annual period, the tendency of the subsoil to yield potassium, and thereby increase the outgo passing from the surface soil, seems to have been augmented beyond any enhancement of its tendency to absorb the potassium passing downward.

Third annual period (tables 7 and 8). During the third annual period the 8-ton surface-soil tanks gave a fairly uniform outgo of potassium and an average practically identical with that of the corresponding subsoil tanks. No outstanding relationship appeared for these two sets of seven each during this period. All of the CaO had become carbonated, considerably prior to the end of the initial annual period, as was pointed out in the discussion of the second year's results from the 8-ton calcium and magnesium oxide and carbonate tanks. In this period, however, the effect of the hydrated lime is distinctly manifested in the case of the 32-ton and 100-ton tanks, in both of which treatments a large part of the applied lime had still remained in the solid hydrate phase. The third year was an exceptionally wet period and the soil was almost continuously subjected to the intense treatment of digestion with solutions of Ca(OH)$_2$. But, if we delete the data obtained from the analyses of the leachings of tanks 8 and 15, in averaging the results from the three batteries, we have 13.7 pounds as an average from the seven 8-ton surface-soil tanks, as against 16.1 pounds and 13.3 pounds, respectively, from the averages derived from leachings obtained from 6 tanks each of the 32-ton and 100-ton treatments. Excepting tanks 8 and 15, in no case has any one of the lime- or magnesia-treated tanks given an outgo of potassium equivalent to that of the blank-treatment. check. There are no consistent or outstanding variations induced by the several treatments, other than the exception above noted; and, if from the average we exclude the data derived from the leachings secured from the two tanks 8 and 15, we have as an average from 19 calcic and magnesic treatments 14.3 pounds as against the 28.2 pounds outgo of the no-treatment tank.

In comparing the surface-soil tanks with the surface-subsoil tanks, we have respective averages of 13.7 pounds and 13.8 pounds for the 8-ton treatments; 16.1 pounds and 16.5 pounds, respectively, for the 32-ton tanks and 13.3 pounds and 15.6 pounds, respectively, for the 100-ton treatments, the 32-ton CaO and the 100-ton CaO tanks being omitted from the latter two averages in the case of the surface soil. Thus, in the treatments other than the two exceptions noted, the underlying subsoil has not determinably altered the potassium salt concentration of the leachings from the surface soil. However, the estopping tendency of the subsoil is distinctly manifested in the case of the 32-ton and 100-ton tanks no. 29 and 36. Assuming the same outgo of potassium from identically treated portions of surface soil with and without underlying subsoil, we find that tank 29 has fixed 31.3 pounds, the difference between 50.4 pounds (tank 8) and 19.1 pounds (tank 29). On the same assumption, the
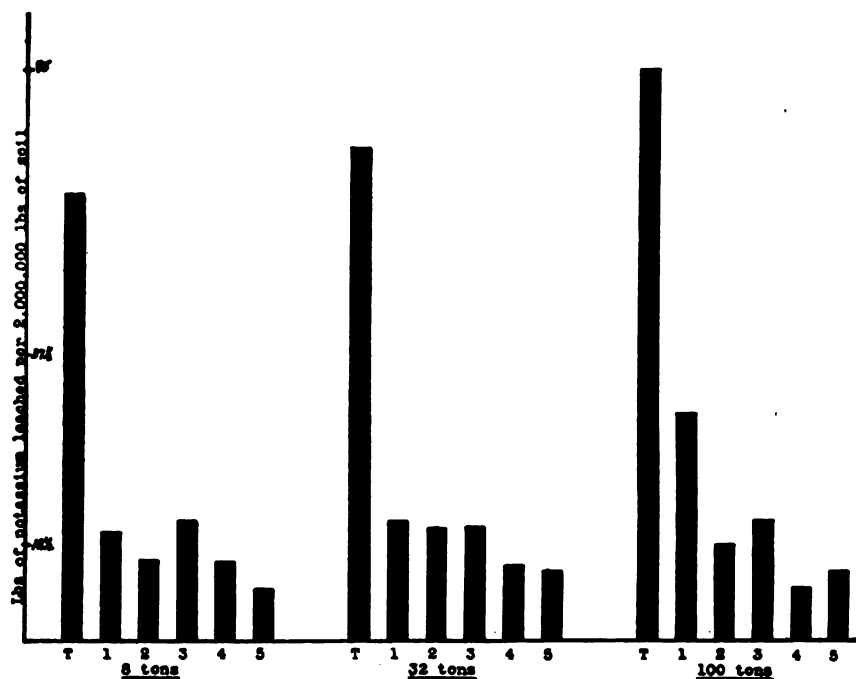
FIG. 11. 100-MESH LIMESTONE TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods
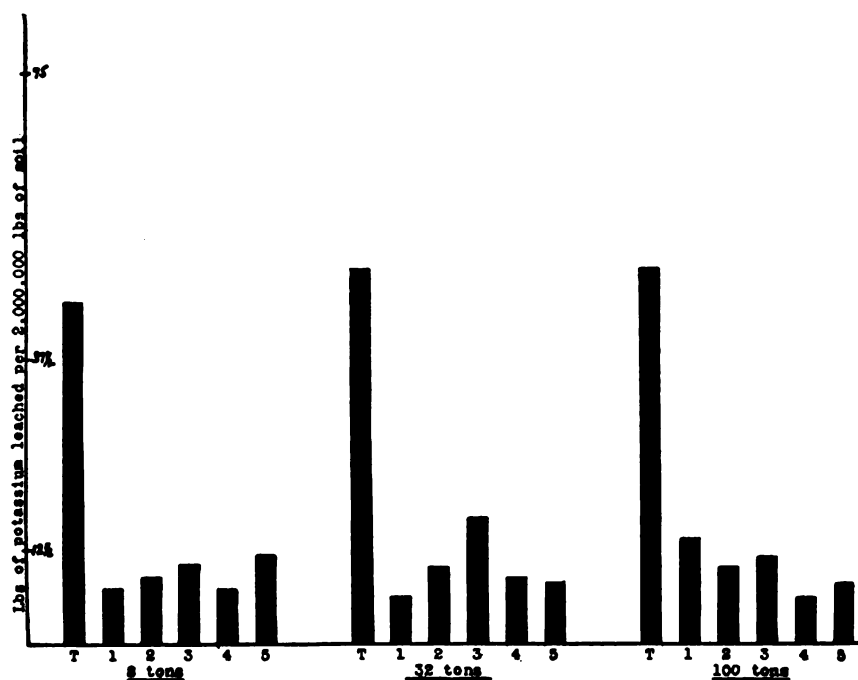
FIG. 12. 100-MESH DOLOMITE TREATMENTS, SURFACE-SOIL-ONLY TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods

TABLE 7

*Amounts of potassium leached from Cumberland loam, surface soil only, in field lysimeters—
July, 1916, to July, 1917*

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and 100 tons per acre 2,000,000 pounds of soil

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS[3] PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with soil	Rate per 2,000,000 pounds of soil				
		tons				
1	CaO	8	170.1	2.17	0.3691	16.3
2	MgO	∞8	172.5	1.40	0.2415	10.7
3	CaCO₃	∞8	165.6	1.71	0.2832	12.5
4	MgCO₃	∞8	193.5	1.38	0.2670	11.8
5	Limestone	∞8	180.1	2.18	0.3926	17.3
6	Dolomite	∞8	139.9	1.69	0.2364	10.4
7	Magnesite	∞8	185.5	2.04	0.3784	16.7
	Average	∞8	172.5	1.80	0.3097	13.7
8	CaO	32	167.0	6.85	1.1439	50.4
9	MgO	∞32	164.4	1.59	0.2614	11.5
10	CaCO₃	∞32	181.6	2.35	0.4268	18.8
11	MgCO₃	∞32	149.5	2.06	0.3080	13.6
12	Limestone	∞32	185.9	2.25	0.4183	18.4
13	Dolomite	∞32	175.3	2.12	0.3716	16.4.
14	Magnesite	∞32	197.3	2.06	0.4064	17.9
	Average	∞32	174.4	2.75	0.4738	21.0
				2.07†	0.3654†	(16.1)†
15	CaO	100	150.7	18.88	2.8452	125.5
16	MgO	∞100	136.2	1.55	0.2111	9.3
17	CaCO₃	∞100	163.7	2.70	0.4420	19.5
18	MgCO₃	∞100	143.2	1.38	0.1976	8.7
19	Limestone	∞100	168.8	1.74	0.2937	13.0
20	Dolomite	∞100	157.3	1.65	0.2595	11.4
21	Magnesite	∞100	172.4	2.33	0.4017	17.7
	Average	∞100	156.0	4.32	0.6644	29.3
				1.89*	0.3009*	(13.3)*
46	Blank		192.5	4.03	0.6404	28.2
Average of all treated tanks			167.6	2.96	0.4826	21.4†
				1.39†*	0.3245†*	(14.3)*

† Average not including tank 8, CaO 32 tons.

* Average not including tank 15, CaO 100 tons.
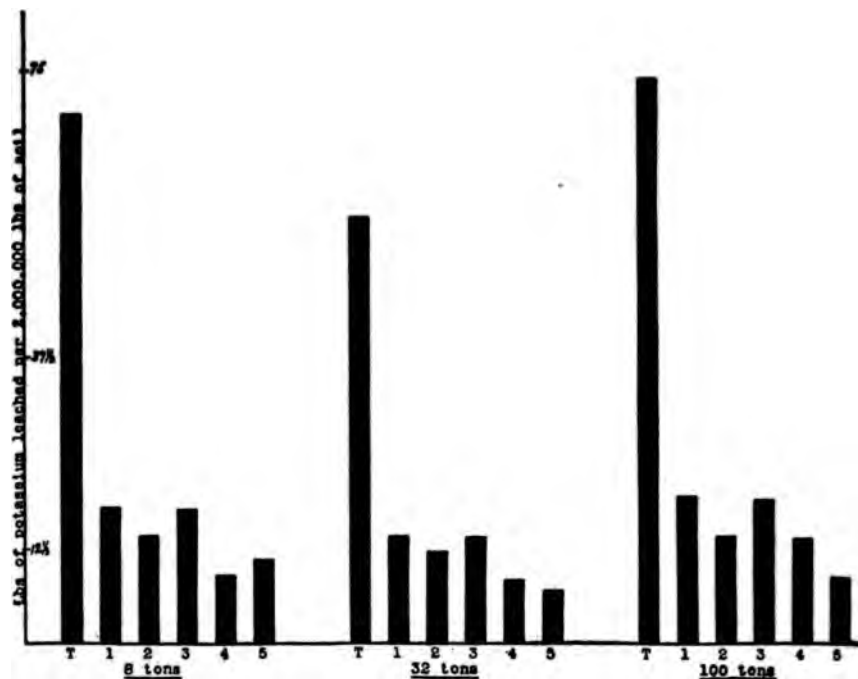
FIG. 13. 100-MESH DOLOMITE TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods

FIG. 14. 100-MESH MAGNESITE TREATMENTS, SURFACE-SOIL-ONLY TANKS
CUMBERLAND LOAM
T = total; numbers refer to annual periods

TABLE 8

Amounts of potassium leached from Cumberland loam, surface soil and subsoil, in field lysimeters— July, 1916, to July, 1917

'reatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and mesh magnesite, each in amounts equivalent to CaO, at the rates of 8 tons, 32 tons and tons per acre 2,000,000 pounds of soil; also Wollastonite and Serpentine at the rate of ons

	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
IK IER	Material mixed with surface soil	Rate per 2,000,000 pounds of soil				
		tons				
	CaO	8	173.7	1.28	0.2223	9.8
	MgO	∞8	169.9	1.37	0.2328	10.3
	CaCO₃	∞8	180.8	1.05	0.1898	8.4
	MgCO₃	∞8	171.1	2.90	0.4202	18.5
	Limestone	∞8	171.3	2.14	0.3665	16.2
	Dolomite	∞8	173.1	2.30	0.3981	17.6
	Magnesite	∞8	162.4	2.22	0.3605	15.9
	Average	∞8	171.7	1.89	0.3129	13.8
	CaO	32	157.3	2.76	0.4341	19.1
	MgO	∞32	154.6	2.56	0.3958	17.5
	CaCO₃	∞32	173.1	2.19	0.3791	16.7
	MgCO₃	∞32	161.0	2.22	0.3574	15.8
	Limestone	∞32	163.2	2.10	0.3427	15.1
	Dolomite	∞32	172.7	1.85	0.3195	14.1
	Magnesite	∞32	186.4	2.12	0.3952	17.4
	Average	∞32	166.9	2.26	0.3748	16.5
	CaO	100	163.1	1.52	0.2479	10.9
	MgO	∞100	163.6	2.40	0.3926	17.3
	CaCO₃	∞100	165.4	1.84	0.3043	13.4
	MgCO₃	∞100	151.8	2.13	0.3233	14.3
	Limestone	∞100	178.0	2.05	0.3649	16.1
	Dolomite	∞100	179.4	2.37	0.4252	18.8
	Magnesite	∞100	183.1	3.14	0.4113	18.2
	Average	∞100	169.2	2.21	0.3528	15.6
	Wollastonite	32	189.0	4.49	0.8486	37.4
	Serpentine	32	183.3	2.65	0.4857	21.4
	Blank		165.5	6.40	1.0592	46.7
	verage of treated tanks except no. 43 and 44.		169.3	2.12	0.3468	15.3

outgo of 125.5 pounds from tank 15 (surface soil only) has been decreased to 10.9 pounds through the absorption of potassium by the subsoil in tank 36. This is equivalent to an estoppage of 114.6 pounds for this annual period. Not only has the subsoil absorbed potassium salts, but all downward moving calcium hydroxide has also been stopped. It would be expected that the leachings from the heavily treated CaO tank would be practically saturated with Ca(OH)$_2$ many times during the period of over 5 years; however, during this period hydroxide has failed to come through the stratum of subsoil.
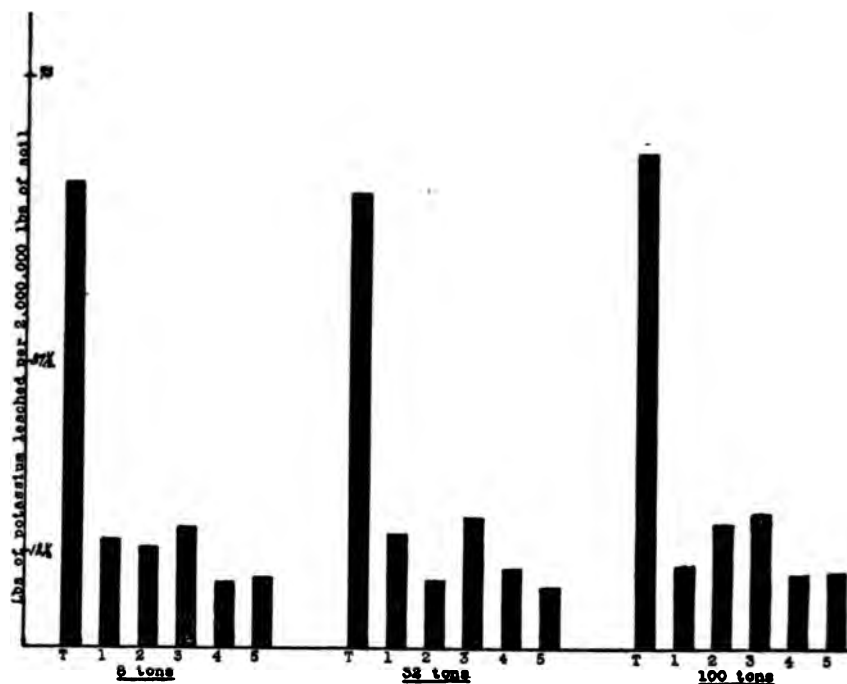
FIG. 15. 100-MESH MAGNESITE TREATMENTS, SURFACE-SOIL-SUBSOIL TANKS,
CUMBERLAND LOAM
T = total; numbers refer to annual periods

Fourth annual period (*tables 9 and 10*). This annual period was very much drier than the preceding one. The restricted opportunity for hydrolysis of the potassic silicates is uniformly demonstrated for all treatments. During this year, very little, if any, hydroxide was present in tank 8, most, if not all, having undergone reversion to carbonate by July, 1917. However, considerable hydroxide still leached from the surface soil containing the 100-ton CaO treatment. The group averages of potassium leachings from the shallow tanks were 6.6 pounds, 7.6 pounds and 7.2 pounds, respectively for the 8-, 32- and 100-ton treatments, tank 15 not being included in the last average. With no. 15 included, the average of the 100-ton equivalent tanks becomes 11.2 pounds

TABLE 9

Amounts of potassium leached from Cumberland loam, surface soil only, in field lysimeters—
July, 1917, to July, 1918

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and
100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and
100 tons per acre 2,000,000 pounds of soil

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with soil	Rate per 2,000,000 pounds of soil				
		tons				
1	CaO	8	81.5	1.42	0.1158	5.1
2	MgO	∞8	70.9	1.88	0.1333	5.9
3	CaCO₃	∞8	86.5	1.98	0.1713	7.6
4	MgCO₃	∞8	89.2	1.51	0.1347	5.9
5	Limestone	∞8	84.1	2.02	0.1699	7.5
6	Dolomite	∞8	75.2	2.16	0.1624	7.2
7	Magnesite	∞8	82.4	2.02	0.1664	7.3
	Average	∞8	81.4	1.86	0.1505	6.6
8	CaO	32	71.9	2.06	0.1481	6.5
9	MgO	∞32	74.3	1.80	0.1337	5.9
10	CaCO₃	∞32	76.8	3.34	0.2565	11.3
11	MgCO₃	∞32	74.9	1.72	0.1288	5.7
12	Limestone	∞32	92.7	1.98	0.1835	8.1
13	Dolomite	∞32	84.6	2.34	0.1980	8.7
14	Magnesite	∞32	90.2	1.84	0.1660	7.3
	Average	∞32	80.8	2.15	0.1735	7.6
15	CaO	100	45.4	17.56	0.7972	35.2
16	MgO	∞100	55.4	2.54	0.1407	6.2
17	CaCO₃	∞100	66.5	2.00	0.1330	5.9
18	MgCO₃	∞100	54.6	1.82	0.0994	4.4
19	Limestone	∞100	68.9	3.18	0.2191	9.7
20	Dolomite	∞100	65.8	2.10	0.1382	6.1
21	Magnesite	∞100	74.3	3.32	0.2467	10.9
	Average	∞100	61.6	4.65 (2.48)*	0.2535 (0.1217)*	11.2 (7.2)*
46	Blank		109.6	3.50	0.3836	16.9
	Average of all treated tanks		74.6	2.89	0.1925	8.5
	Average of treated tanks, except no. 15			2.16	0.1486	7.1

* Average not including tank 15.

TABLE 10

Amounts of potassium leached from Cumberland loam, surface soil and subsoil, in field lysimeters— July, 1917, to July, 1918

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and 100 tons per acre 2,000,000 pounds of soil; also wollastonite and serpentine at the rate of 32 tons

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with surface sol	Rate per 2,000,000 pounds of soil				
		tons				
22	CaO	8	89.5	1.82	0.1629	7.2
23	MgO	∞8	85.3	2.98	0.2542	11.2
24	CaCO₃	∞8	82.0	2.84	0.2329	10.3
25	MgCO₃	∞8	84.5	1.96	0.1656	7.3
26	Limestone	∞8	84.7	2.90	0.2456	10.8
27	Dolomite	∞8	83.8	2.38	0.1994	8.8
28	Magnesite	∞8	68.4	2.46	0.1683	7.4
	Average	∞8	82.6	2.48	0.2041	9.0
29	CaO	32	75.9	3.34	0.2535	11.2
30	MgO	∞32	88.6	2.54	0.2250	9.9
31	CaCO₃	∞32	85.3	2.66	0.2269	10.0
32	MgCO₃	∞32	102.9	2.20	0.2264	10.0
33	Limestone	∞32	86.2	2.60	0.2241	9.9
34	Dolomite	∞32	84.6	2.26	0.1911	8.4
35	Magnesite	∞32	96.1	2.49	0.2393	10.6
	Average	∞ 32	88.5	2.58	0.2266	10.0
36	CaO	100	82.4	2.68	0.2208	9.7
37	MgO	∞100	75.3	1.90	0.1431	6.3
38	CaCO₃	∞100	80.2	2.16	0.1732	7.6
39	MgCO₃	∞100	74.4	1.32	0.0982	4.3
40	Limestone	∞100	88.3	1.86	0.1642	7.2
41	Dolomite	∞100	82.4	3.76	0.3098	13.7
42	Magnesite	∞100	94.1	2.42	0.2277	10.0
	Average	∞100	82.4	2.30	0.1910	9.5
43	Wollastonite	32	89.8	2.02	0.1814	8.0
44	Serpentine	32	103.3	3.27	0.3378	14.9
45	Blank		69.2	5.28	0.3654	16.1
Average of treated tanks except no. 43 and 44			84.5	2.45	0.2072	9.5

instead of 7.2 pounds. The surface-soil tanks gave a grand average of 8.5 pounds as compared with 9.5 pounds for the surface-soil-subsoil tanks. The liberation and leaching of potassium was still evidenced in the case of the 100-ton tank no. 15, although the outgo was less than in the more humid preceding annual period. The function of the subsoil in absorbing the potassium liberated and leached from the surface soil was again manifested. The average loss of potassium from the 21 treated surface-soil tanks was only about one-half that of the blank. The same was true in the case of the comparison between the treated surface-soil-subsoil tanks and their blank. During this period, as in the preceding annual periods, the increase in the rate of treatments induced no augmentation in the potassium outgo, except in the case of burnt lime at the rate of 100 tons.

Fifth annual period (*tables 11 and 12*). Again in this period there is effected no consistent difference in potassium outgo to be attributed to variation in form or amount of treatment, for either the shallow or deep tanks, except once more in the case of the 100-ton CaO treatment and possibly also in the apparent parallel between the increase of $MgCO_3$ treatment and depression of potassium leachings therefrom. Here again the potassium outgo from the deep tank which received burnt lime at the rate of 100 tons per acre is decidedly less than in the case of the corresponding shallow tank. The continued tendency of both lime and magnesia treatments to effect restriction of potassium outgo is evidenced by the diminished amounts of potassium leached from the treatments, as compared with that from the untreated soil. This holds true for both depths. The analyses of both shallow and deep tank leachings for salts of calcium and magnesium demonstrate that the surface soil has yielded large amounts of the salts of these two elements to the subsoil and that the subsoil has stopped and in most cases still stops the salts yielded to it by the surface soil. Hence, it would be expected that the parallel of depressed potassium outgo and the continued occurrence of solutions of lime and magnesia salts—with their probably or possibly continued absorption by the surface soil—would have been transmitted ere this in some measure also to the subsoil.

Summary of potassium outgo data secured during the 5-year period. The results of the 5-year period are shown graphically for each annual period and for the total period in figures 1 to 15, inclusive. All the figures, except figure 2, are in the same scale. The heavy outgo of potassium from the two heavy CaO surface-soil tanks necessitated the adoption of the different scale used in this particular figure.

Considering tables 13 and 14, which embody the summation of the data obtained during the 5-year period, we find a very uniform outgo of potassium irrespective of form or amount of treatment, save only in the instances of the two heavier burnt lime treatments. The average potassium outgo from the shallow tanks is very close to that found in the case of the deep tanks.

The losses of potassium from tanks 43 and 44, which were treated with the more insoluble forms of lime and magnesia, wollastonite and serpentine, ap-

TABLE 11

Amounts of potassium leached from Cumberland loam, surface soil only, in field lysimeters—July, 1918, to July, 1919

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and 100 tons per acre 2,000,000 pounds of soil

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with surface soil	Rate per 2,000,000 pounds of soil				
		tons				
1	CaO	8	112.1	1.53	0.1715	7.6
2	MgO	∞8	116.1	2.18	0.2531	11.2
3	CaCO₃	∞8	121.3	1.57	0.1904	8.4
4	MgCO₃	∞8	119.4	1.59	0.1898	8.4
5	Limestone	∞8	118.4	1.71	0.2025	8.9
6	Dolomite	∞8	115.8	2.25	0.2606	11.5
7	Magnesite	∞8	118.7	2.35	0.2789	12.3
	Average	∞8	116.8	1.88	0.2209	9.8
8	CaO	32	105.2	2.33	0.2451	10.8
9	MgO	∞32	107.7	1.56	0.1680	7.4
10	CaCO₃	∞32	115.9	1.52	0.1762	7.8
11	MgCO₃	∞32	114.0	1.08	0.1231	5.4
12	Limestone	∞32	127.5	1.41	0.1798	7.9
13	Dolomite	∞32	116.1	1.55	0.1800	7.9
14	Magnesite	∞32	120.4	1.51	0.1818	8.0
	Average	∞32	115.3	1.56	0.1791	7.9
15	CaO	100	88.3	15.25	1.3466	59.4
16	MgO	∞100	103.6	2.51	0.2600	11.5
17	CaCO₃	∞100	111.5	1.66	0.1851	8.2
18	MgCO₃	∞100	103.7	0.83	0.0861	3.8
19	Limestone	∞100	114.2	1.81	0.2067	9.1
20	Dolomite	∞100	110.6	1.64	0.1814	8.0
21	Magnesite	∞100	116.6	1.29	0.1504	6.6
	Average	∞100	106.9	3.57 (1.62)*	0.3452 (0.1783)*	15.2 (7.9)*
46	Blank		115.9	2.49	0.2886	12.7
Average of all treated tanks			113.2	2.67	0.2903	12.8
Average of treated tanks except no. 15				1.69	0.1929	8.5

* Average not including tank 15, CaO 100 tons.

TABLE 12

Amounts of potassium leached from Cumberland loam, surface soil and subsoil, in field lysimeters—July, 1918, to July, 1919

Treatments of CaO, MgO, CaCO₃, MgCO₃, 100-mesh limestone, 100-mesh dolomite and 100-mesh magnesite, each in amounts equivalent to CaO at the rates of 8 tons, 32 tons and 100 tons per acre; also wollastonite and serpentine at the rate of 32 tons

TANK NUMBER	TREATMENT		TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Material mixed with surface soil	Rate per 2,000,000 pounds of soil				
		tons				
22	CaO	8	121.3	1.45	0.1759	7.8
23	MgO	∞8	116.1	1.29	0.1498	6.6
24	CaCO₃	∞8	124.9	1.70	0.2123	9.4
25	MgCO₃	∞8	116.2	1.04	0.1208	5.3
26	Limestone	∞8.	117.8	1.36	0.1602	7.1
27	Dolomite	∞8	120.1	2.04	0.2450	10.8
28	Magnesite	∞8	119.0*	1.75	0.2083	9.2
	Average	∞8	119.3	1.52	0.1817	8.0
29	CaO	32	110.6	2.70	0.2986	13.2
30	MgO	∞32	116.1	1.27	0.1474	6.5
31	CaCO₃	∞32	119.3	1.67	0.1992	8.8
32	MgCO₃	∞32	119.8	1.16	0.1389	6.1
33	Limestone	∞32	123.5	1.73	0.2136	9.4
34	Dolomite	∞32	120.5	1.36	0.1639	7.2
35	Magnesite	∞32	125.3	1.42	0.1779	7.8
	Average	∞32	119.3	1.61	0.1913	8.4
36	CaO	100	105.6	2.24	0.2365	10.4
37	MgO	∞100	111.0	1.59	0.1765	7.8
38	CaCO₃	∞100	121.8	1.26	0.1535	6.8
39	MgCO₃	∞100	120.9	0.92	0.1112	4.9
40	Limestone	∞100	123.1	1.64	0.2019	8.9
41	Dolomite	∞100	112.9	1.69	0.1908	8.4
42	Magnesite	∞100	128.9	1.83	0.2359	10.4
	Average	∞100	117.7	1.60	0.1866	8.2
43	Wollastonite	32	121.8	1.97	0.2406	10.6
44	Serpentine	32	137.7	2.06	0.2836	12.5
45	Blank		130.4	3.12	0.4068	18.2
	Average of treated tanks except no. 43 and 44		118.8	1.58	0.1865	8.2

* Leak sprung in outlet, average volume of tanks 26 and 27 used.

TABLE 13

Summary of potassium leaching data, Cumberland loam surface soil only, for the 5-year period, July, 1914 to July, 1919

TANK NUMBER	Material mixed with soil	Rate per 2,000,000 pounds of soil	LITRES LEACHED		PARTS PER MILLION IN SOLUTION K		GRAMS PER TANK		POUNDS FROM ACRE SURFACE 2,000,000 POUNDS OF SOIL	
			Total	Average annual	Total	Average annual	Total	Average annual	Total	Average annual
		loss								
1	CaO	8	458.1	91.6	12.09	2.42	1.2761	0.2552	56.3	11.3
2	MgO	∘8	542.6	108.5	10.86	2.17	1.0551	0.2110	48.8	9.8
3	CaCO₃	∘8	564.5	112.9	14.21	2.84	1.3722	0.2744	60.6	12.1
4	MgCO₃	∘8	623.1	124.6	11.15	2.23	1.2462	0.2492	55.0	11.0
5	Limestone	∘8	593.8	118.8	12.02	2.40	1.2975	0.2595	57.3	11.5
6	Dolomite	∘8	505.8	101.2	10.35	2.07	1.0181	0.2036	44.9	9.0
7	Magnesite	∘8	590.6	118.2	10.06	2.01	1.1952	0.2390	52.7	10.5
	Average	∘8	554.1	110.8	11.53	2.31	1.2086	0.2417	53.7	10.7
8	CaO	32	537.8	107.6	17.84	3.57	2.1426	0.4285	94.4	18.9
9	MgO	∘32	529.3	105.9	8.49	1.70	0.8879	0.1776	39.1	7.8
10	CaCO₃	∘32	571.8	112.4	14.21	2.84	1.5422	0.3084	68.0	13.6
11	MgCO₃	∘32	494.3	98.9	8.65	1.73	0.8375	0.1675	36.9	7.4
12	Limestone	∘32	612.4	122.5	9.36	1.87	1.1486	0.2297	50.6	10.1
13	Dolomite	∘32	581.2	116.2	9.55	1.91	1.1144	0.2229	49.1	9.8
14	Magnesite	∘32	627.3	125.5	8.93	1.79	1.1215	0.2243	49.4	9.9
	Average	∘32	564.9	113.0	11.00	2.20	1.2564	0.2513	55.4	11.1

15	CaO............	100	471.0	94.2	58.99	11.80	5.6661	1.1322	249.8	49.9
16	MgO............	<100	464.6	92.9	11.31	2.26	1.0026	0.2005	44.2	8.8
17	CaCO₃...........	<100	514.3	103.5	12.36	2.47	1.2993	0.2599	57.3	11.5
18	MgCO₃..........	<100	459.5	91.9	9.94	1.99	0.8630	0.1726	38.1	7.6
19	Limestone.......	<100	555.6	111.9	11.44	2.29	1.1989	0.2398	52.9	10.6
20	Dolomite........	<100	506.7	101.3	12.77	2.55	1.1341	0.2268	49.9	10.0
21	Magnesite.......	<100	567.6	113.5	11.16	2.23	1.2528	0.2506	55.2	11.0
	Average........	<100	505.6 (511.4)*	101.3 (102.5)*	18.28 (11.50)*	3.66 (2.30)*	1.7738 (1.1251)*	0.3546 (0.2250)*	78.2 (48.3)*	15.6 (9.9)*
46	Blank		418.0†	139.3†	10.02†	3.34†	1.3126†	0.4375†	57.8†	19.3†
	Average of all treated tanks........		541.5	108.4	13.60	2.79	1.4129	0.2909	62.4	12.5
	Average of treated tanks except no. 15....		545.0	109.0	11.34	2.27	1.2027	0.2360	53.1	10.6

† Average for 3 years.
* Average not including tank 15, CaO 100 tons.

TABLE 14

Summary of potassium leaching data, Cumberland loam surface soil and subsoil, for the 5-year period, July, 1914 to July, 1919

TANK NUMBER	TREATMENT Material mixed with surface soil	Rate per 2,000,000 pounds of soil	LITRES LEACHED		PARTS PER MILLION IN SOLUTION K		GRAMS PER TANK		POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL	
			Total	Average annual	Total	Average annual	Total	Average annual	Total	Average annual
		less								
22	CaO	8	579.5	115.9	12.68	2.54	1.2843	0.2569	56.7	11.3
23	MgO	<>8	555.2	111.0	13.67	2.73	1.3048	0.2610	57.6	11.5
24	CaCO₃	<>8	584.7	116.9	11.66	2.33	1.1918	0.2384	52.7	10.5
25	MgCO₃	<>8	541.1	108.2	17.18	3.44	1.3394	0.2679	59.0	11.8
26	Limestone	<>8	551.1	110.2	13.72	2.74	1.3544	0.2709	58.9	11.8
27	Dolomite	<>8	563.5	112.7	15.85	3.07	1.5595	0.3119	68.9	13.8
28	Magnesite	<>8	553.5	110.7	13.05	2.61	1.3602	0.2720	59.9	12.0
	Average	<>8	561.2	112.4	13.90	2.78	1.3421	0.2684	59.1	11.8
29	CaO	32	525.9	105.2	17.36	3.47	1.7552	0.3510	77.4	15.5
30	MgO	<>32	530.0	106.0	16.33	3.27	1.5734	0.3147	69.4	13.9
31	CaCO₃	<>32	560.4	112.1	14.24	2.85	1.5800	0.3160	69.7	13.9
32	MgCO₃	<>32	577.9	115.6	13.90	2.78	1.5803	0.3161	66.0	13.2
33	Limestone	<>32	553.5	110.7	14.48	2.90	1.4709	0.2942	64.8	13.9
34	Dolomite	<>32	569.7	113.9	12.09	2.42	1.2699	0.2540	55.9	11.2
35	Magnesite	<>32	605.7	121.1	11.82	2.36	1.3536	0.2707	59.7	11.9
	Average	<>32	560.4	112.1	14.32	2.86	1.5119	0.3024	66.1	13.2

36	CaO	100	560.1	112.0	12.93	2.59	1.3482	0.2696	59.3	11.9
37	MgO	100	546.6	109.3	13.49	2.70	1.4520	0.2904	64.0	12.8
38	CaCO₃	100	526.4	105.3	12.05	2.41	1.0921	0.2184	48.1	9.6
39	MgCO₃	100	495.9	99.2	8.72	1.74	0.8883	0.1777	39.2	7.8
40	Limestone	100	598.8	119.8	15.51	3.10	1.7089	0.3418	75.3	15.1
41	Dolomite	100	581.5	116.3	15.79	3.16	1.6801	0.3360	74.1	14.8
42	Magnesite	100	583.0	116.6	14.69	2.94	1.5038	0.3008	66.4	13.3
	Average	100	556.0	111.2	13.31	2.66	1.3819	0.2764	60.9	12.2
43	Wollastonite	32	615.9	123.2	16.80	3.36	2.0933	0.4187	92.3	18.5
44	Serpentine	32	646.1	129.2	13.95	2.75	1.8035	0.3607	79.5	15.9
45	Blank*		365.1*	121.7	14.80*	4.93	1.8314*	0.6105	81.0*	27.0*
	Average of treated tanks—except no. 43 and 44		559.2	111.9	13.84	2.77	1.4120	0.2824	62.0	12.4

* Average for 3 years only.

proach more nearly the average outgo of the checks than do the more soluble
precipitated and mineral carbonates. These two deep tanks might be con-
sidered as near-blanks. However, the calcium silicate of wollastonite readily
undergoes hydrolysis; but the magnesium silicate complex of serpentine is
very stable. Therefore, it may be that such an intermediate result is a true
indication of some, though restricted, tendency toward retardation of potassium
outgo because of calcium or magnesium salts engendered by the hydrolysis of
the two siliceous materials.

Disregarding, for the present, the heavy burnt lime treatments of tanks 8
and 15, the results may be considered as indicating either of two possibilities;
(1) the calcic and magnesic materials may have actually depressed the tendency
of the native soil potassic compounds to undergo hydrolysis; or (2) the calcic
and magnesic materials may have accelerated the hydrolysis of the potassic
complexes, at the same time imparting to the soil an enhanced tendency to
reabsorb or readsorb such potassium salts as were liberated.

Considering the first hypothesis, we find, as is demonstrated by examination
of the tank leachings data and also by the analysis of the soil atmospheres,
that the larger applications of calcic and magnesic materials, including car-
bonates, tend to withdraw from the soil not only the CO_2 present in free soil
moisture, but also large additional amounts which were, in the case of the
several carbonate treatments, apparently condensed upon the surface of soil
particles. A large number of periodic double titrations of the leachings from
each tank have demonstrated that enough of calcium or magnesium carbonate
is usually present to account for the presence of all dissolved CO_2 as being in
bicarbonate formations. Thus, the bathing soil solutions become impreg-
nated with the bicarbonates of calcium or magnesium where such carbonate
treatments are given. On the other hand, we find more atmospheric CO_2 in
the untreated tank than in the treated ones. We also find that the soil solu-
tion bathing the soil particles of the untreated soil is to a greater extent com-
posed of the hypothetical acid H_2CO_3. The native soil is acid and free of
determinable carbonates; however, the leachings from the blank are always alka-
line to methyl orange and they are generally impregnated with determinable
amounts of calcium bicarbonate, derived from the action of the carbonated
water of the soil upon native calcic materials. It would therefore seem that
the carbonated water, acting upon a relatively small amount of native calcic
siliceous compounds, would be ordinarily unsaturated with reference to lime or
magnesia, or, that it would at least become impregnated therewith much more
slowly than in the case of the calcic and magnesic treatments; and that the
additional uncombined carbonated water would then proceed to hydrolyze the
less readily hydrolyzable potassic silicate complexes.

Thus we come to compare the activity of H_2CO_3 with the activity of
CaH_2CO_3 and that of MgH_2CO_3 in effecting the hydrolysis of native potassic
silicates under field exposures in the lysimeters. The results of Plummer (27)
demonstrated that, under laboratory conditions, the presence of bicarbonate

of lime in water solution induced no increase in the liberation of potassium from several finely ground potassic siliceous minerals common to soils. This is in harmony with our findings.

The analyses of the periodic leachings demonstrate that any increase in the amounts of dissolved $CaCO_3$ is not accompanied by any increase in potassium outgo in the free soil-water, such as would be expected, were $CaCO_3$ solutions to effect a liberation of potassium.

Furthermore, the relative activities of the bicarbonates of calcium and magnesium throw some light upon the question. The solubility of $MgCO_3$, as the bicarbonate in carbonated water solutions, is many times that of calcium carbonate in the same solvent, under conditions of normal temperature and pressure. However, although the amounts of the two alkali-earth carbonates applied were of chemical equivalence, there proved to be a wide disparity between the lime-absorption and the magnesia-absorption co-efficients of this soil. This disparity resulted in the rapid absorption and disappearance of the 8-ton-CaO-equivalent treatments of $MgCO_3$, although the major portion of the equivalent $CaCO_3$ application remained as such; so that the comparison between the effects of CaH_2CO_3 and MgH_2CO_3 will be considered as referring more particularly to the two heavier treatments. The active mass of the magnesium bicarbonate solution is much greater than the active mass offered by the calcium carbonate within a given period; hence, were the bicarbonate of lime to effect liberation of potassium, the bicarbonate of magnesium would be expected to accomplish still greater liberation. But, we find. the reverse to be true. The magnesium carbonate treatments have appeared to depress the outgo of potassium salts to an even greater extent than did the treatments of calcium carbonate. Yet, the magnesium carbonate treatments have yielded such concentrated free soil-water solutions of magnesium bicarbonate, that these treatments have been the first to satisfy the absorptive properties of the subsoil and the first to yield concentrated leachings through the subsoil; this, too in spite of the large amount of magnesia absorbed by the soil, as compared with the distinctly smaller amounts of lime absorbed from chemically equivalent amounts of $CaCO_3$. This extensive absorption has taken place directly and without any liberation or exchange of potash. Thus we find that free H_2CO_3 will effect the maximum liberation of potassium to the free-soil water leachings; that the smaller amounts of calcium dissolved in the H_2CO_3 will show some depression in the solvent action and that the larger amounts of magnesium dissolved in the H_2CO_3 will effect a still further depression.

The work of Parker (25) demonstrated that, by the addition of a hydrate solution, the neutralization of acid as it is engendered by the absorption of the strong base from a practically undissociated neutral salt will preclude the evidence of liberation of potassium from the native siliceous materials. In like manner, after the absorption of calcium from a heavy treatment of its carbonate forms, followed by the liberation of the CO_2, this liberated gas will, with water and the excess of $CaCO_3$, form a nearly neutral salt which would be

expected to be less active than the free hypothetical acid H_2CO_3. While this would be true of heavy calcic treatments, the reverse would apply in the case of light treatments. For, were all of the added $CaCO_3$ to undergo absorption and disintegration, the liberated CO_2 would to some extent enrich the free soil-water and afford a stronger H_2CO_3 solution. However, this concentration of H_2CO_3 is in turn reduced by the reversal of the reaction induced by the hydrolysis of the newly formed absorption compounds of calcium. Furthermore, the analyses of leachings and soil atmospheres of the tanks indicated a selective condensation of CO_2, which inhibits any extensive outgo of CO_2 dissolved in the low-calcium content free water of the light treatment or no-treatment tanks. But, in the presence of excessive amounts of the more soluble carbonate forms, particularly magnesium carbonate, such condensed CO_2 will readily combine with the carbonate and with it come from the soil in the bicarbonate combinations.

In the light of such data, it would seem reasonable and logical to deduce that the dissolving of calcium and magnesium carbonate by the carbonated soil-water has depressed the solvent action of the soil-water thus impregnated; i.e., the application of the precipitated and native mineral carbonates has depressed the amounts of potassium yielded to the free soil-water through any hydrolytic disintegration of the native silicates containing potassium.

Nevertheless, on the other hand, *we have the second hypothesis* whereby it is assumed that actual liberation of water-soluble potassium has resulted from additions of calcic and magnesic compounds, but that such treatment has likewise enhanced the absorptive tendency of the soil, thus permitting reabsorption or readsorption of the supposedly liberated potassium. The tendency of soils to absorb potassium from neutral potassic salts is well known. The extent of absorption of the cation and liberation of the anion is dependent, in part, upon the degree of dissociation of the dissolved neutral salt. However, following such absorption, we find a tendency toward attainment of a dynamic equilibrium, which results in a subsequent augmentation in the amounts of soluble or extractable potassium to be found in the free soil-water.

Shreiner and Failyer (30), in demonstrating the absorption of potassium from neutral salts, also show the tendency toward subsequent release of the absorbed potassium to leaching. Initially, the leachings were of maximum potassium salt concentration, decreasing to a near-constant. However, save for the exception noted, CaO at the rates of 32 tons and 100 tons, our lysimeter leachings from the large number of precipitated and native mineral carbonates do not evidence any diffusion of soil-surface concentrates of potassic salts to the free soil-water, such as would be anticipated had there occurred an enrichment of soluble potassium salts in the soil-particle surface film, through the phenomenon of interchange of bases. On the other hand, the eventual increased outgo of potassium—the exceptions which transpired in the case of the heavy burnt lime treatments of tanks 8 and 15—is in conformity with the above-cited demonstrations of the delayed tendency of liberated potassium salts to

enter free soil-water solution, upon being subjected to leaching. According to Shreiner and Failyer (30) "the absorbed potassium, like the absorbed phosphate, is continually diffusing into the free soil-moisture and becoming, therefore, directly accessible to plants." As a matter of fact, not only did all of the chemical and mineral carbonates induce a diminished leaching of potassium, but the 8-ton CaO treatments, which speedily reverted to the carbonate, also failed to show any increase, but rather a decrease of potassium outgo during the 5-year period; while *the hydrate still present in the 32-ton and 100-ton treatments showed no indication of liberation of potassium until the third and humid year.*

In advancing the second hypothesis, that of reabsorption of any liberated potassium, it might be contended that the liberation of potassium actually found in the two heavy CaO surface-soil tanks was due to a coincidental granulation of the soil of such nature as to preclude reabsorption; while, in the case of the other treatments of calcic and magnesic compounds, potassium liberation was effected without parallel granulation and vitiation of the soil's powers of absorption. But, since the physical change of soil structure induced by the 32-ton and 100-ton burnt-lime treatments was immediate, while the release of increased amounts of potassium to leachings was deferred for over two years, it seems more reasonable to conclude that the disintegration of potassic complexes was induced solely by the burnt lime treatments and by them only under conditions involving intense treatment and long-continued contact. The intensity of treatment found to be requisite to register potassium liberation is of such magnitude as to be well beyond the bounds of practical consideration. Because of the foregoing data, and their relationship to the cited findings of Shreiner and Failyer (30) and those of Plummer (27), we may attribute to the several carbonate treatments an actual depression of hydrolysis and liberation of native soil potassium, rather than any release of potassium through basic exchange.

One further possibility suggests itself. It might be assumed that the chemical and mineral carbonates effected an actual chemical disintegration of the native potassic silicates and that there was induced a resultant absorption of such a nature as to withstand any tendency toward diffusion to the free soil-water derived from natural precipitation; but, that the reabsorbed potassium would, nevertheless, yield to the more intensive activities of plant roots enough potassium to appreciably enhance the supply that would have been obtained without the alkali-earth treatments. Assuming, however, that the plant root-system of a wheat crop would be sufficiently extensive to derive such a hypothetical benefit, the above offered supposition would be invalidated by the previously cited wheat-ash analyses offered by Gaither (12), and by the writer (20). Furthermore, the averages of the annual analyses of corn, oats, wheat, and grass offered by Lyon and Bizzell (18) show that the potassium content of these crops is uninfluenced by applications of 3000 pounds of burnt lime per acre; and that the potash content of those crops is independent of the

amounts of potassium leached, although the plants elaborated about twice as much potassium as was lost from the soils by leaching.

Certain unpublished data were secured by Prof. C. A. Mooers from carefully controlled plat experiments, at the Tennessee station, with the same acid Cumberland loam as that which was placed in the tanks. These data afford an opportunity for observations relative to the dependence to be placed upon lime, as an indirect source of potassium for growing plants. The experiments involve a continuous cowpea-wheat rotation, one crop each of cowpeas and wheat being harvested each year. At the beginning of the experiment and again after a period of 12 years, one ton of burnt lime per acre was applied to certain of the plats, all of which received annual applications of phosphate, both with and without potash. After several years, both crops manifested a distinct need of potassium in the check plats and also in those plats which received phosphate, both with and without lime. The annual applications of phosphate, of course, carried some $CaSO_4$ and the 1-ton application of burnt lime was annually supplemented with calcium to that extent. The second application of burnt lime, like the first, did not correct this distinct need of potassium. On the other hand, an annual application of manure, at the rate of 4 tons per acre, maintained the yields of both crops and prevented any physiological evidence of potassium deficiency. The incorporation of the organic matter of the manure would be followed of course by the formation of large amounts of CO_2 and the enrichment of the soil solution in this product of oxidation. Assuming the potassium content of the manure to be insufficient to offset the insufficiency of potassium registered by both crops, this, in conformity with the lysimeter results, tends to demonstrate that a free soil-water solution of the hypothetical acid H_2CO_3 is more active in the liberation of native soil potassium as a nutrient to the plant, than is the carbonic acid solution of the bicarbonate salt. Following the application of lime or magnesia in their several forms, such occurrences of CaH_2CO_3 or MgH_2CO_3 in the soil-water would be increased, with the strongly indicated tendency toward decreased extraction of potassium. Furthermore, analyses of some of the earlier crops of wheat, which were harvested before the applied lime had been greatly diminished by leaching and through removal of cowpeas and wheat, indicated rather forcibly that the use of lime had appreciably depressed the potassium content of the wheat straw.

The potassium in leachings from the 22 lime-magnesia-sulfur tanks of Cherokee sandy loam during the initial two years

The results contained in tables 15 and 16, and summarized in table 17, are from the Cherokee sandy loam, over a 2-year period of exposure. The annual and aggregate amounts of potassium leached are shown graphically in figures 11 to 19, inclusive. This Cherokee sandy loam is a reddish-brown soil and carries considerable clay. Its total potassium content and that portion soluble

in each N/5 HNO₃ and HCl (Sp. g., 1.115), are given in table 2. The supplementary experiment with this soil was intended primarily as an adjunct to certain observations made in a preliminary report by the writer and associates (22) on the influence of various forms of lime and magnesia upon the outgo of native sulfur as sulfates. This series of tanks, however, embraces a number which have received sulfur in several forms, both with and without lime or magnesia. Since the function of the subsoil was so well demonstrated in the system of 46 tanks, all of the 22 additional tanks were filled with the surface soil only. The series was comprised as follows: one blank and the following no-sulfur single CaO and MgO treatments per 2,000,000 pounds of soil; one receiving 2000 pounds of CaO; one 3750 pounds of CaO; one receiving MgO chemically equivalent to 2000 pounds of CaO; one MgO chemically equivalent to 3750 pounds of CaO; one ground limestone, and one ground dolomite, both of 100-mesh fineness, and each chemically equivalent to 2000 pounds of CaO. Five tanks received sulfur as FeSO₄, at the equivalent rate of 1000 pounds of sulfur per acre 2,000,000 pounds of soil. One of these five sulfate tanks received no additional treatments; one an additional treatment of 3750 pounds of CaO; another an additional application of MgO equivalent to CaO at the rate of 3750 pounds; while the remaining two received, in one case, CaO at the rate of 32 tons and in the other case MgO at the rate chemically equivalent to 32 tons of CaO. In like manner, each tank of another set of five tanks received iron pyrites in an amount furnishing sulfur at the rate of 1000 pounds per 2,000,000 pounds of soil. As in the case of the five sulfate tanks, one received no alkali-earth treatment, while the remaining four were treated singly with the four respective applications of CaO and MgO at the two rates. In still another set of five tanks, powdered sulfur was added to each tank at the rate of 1000 pounds per 2,000,000 pounds of soil. In this set, one tank received sulfur alone, while one each of the other four received CaO or MgO at the two rates. The CaO at the rate of 3750 pounds per 2,000,000 pounds of soil and its chemical equivalence of MgO used in one pair of the checks and in two tanks of each sulfur-treated set, represented an application of 2000 pounds of CaO or its MgO equivalence, plus 1750 pounds of CaO, or its MgO equivalence; the plus treatment representing the amount required to neutralize the immediate acidity of the ferrous sulfate or the potential acidity to be accredited to the unoxidized pyrites and elemental sulfur, in case the latter two materials should undergo complete oxidation. Therefore, of the five tanks which received FeSO₄, with and without lime or magnesia, the four receiving CaO and MgO may be considered as involving treatments of varying amounts of CaSO₄ or MgSO₄. The reaction of the leachings have demonstrated the absence of FeSO₄, therefrom, even in the case of the check; and the leachings have been alkaline to methyl orange without exception, though the original soil was acid in character. As in the case of the 46 lysimeters embracing Cumberland loam, all of the treatments were mixed throughout the 8-inch depth of moist soil, equivalent in each case to 100 pounds of moisture-free soil. The
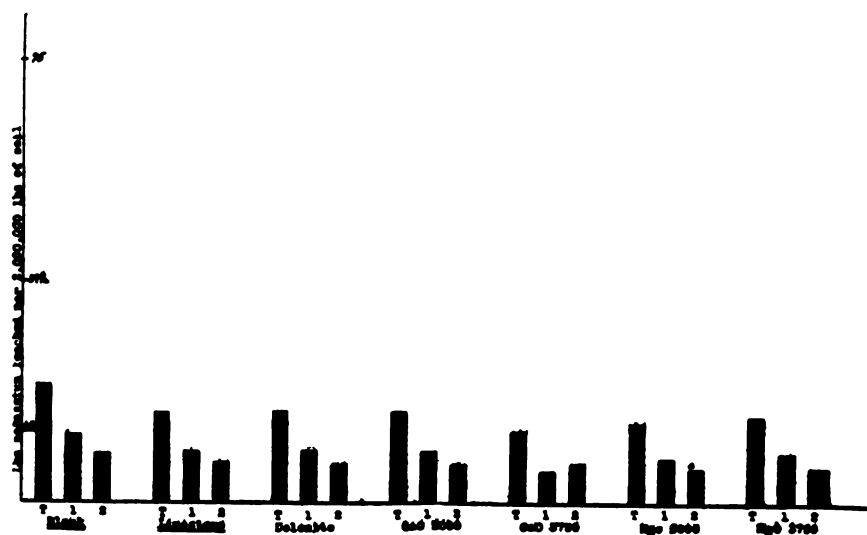
FIG. 16. BLANKS AND CHECKS; CHEROKEE SANDY LOAM, SURFACE SOIL ONLY
T = total; numbers refer to annual periods
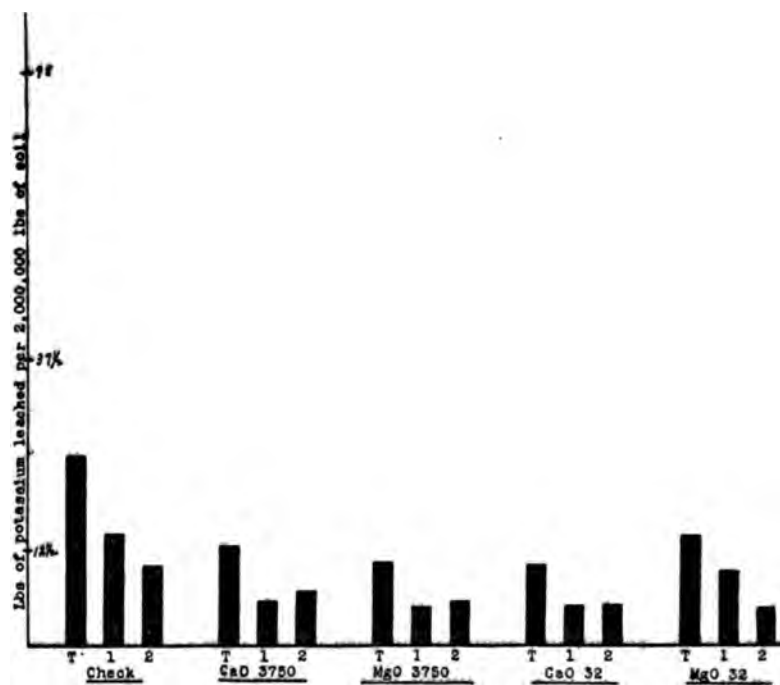
FIG. 17. SULFUR TREATMENTS, FeSO₄ = 1000 POUNDS SULFUR PER 2,000,000
POUNDS CHEROKEE SANDY LOAM, SURFACE SOIL ONLY
T = total; numbers refer to annual periods
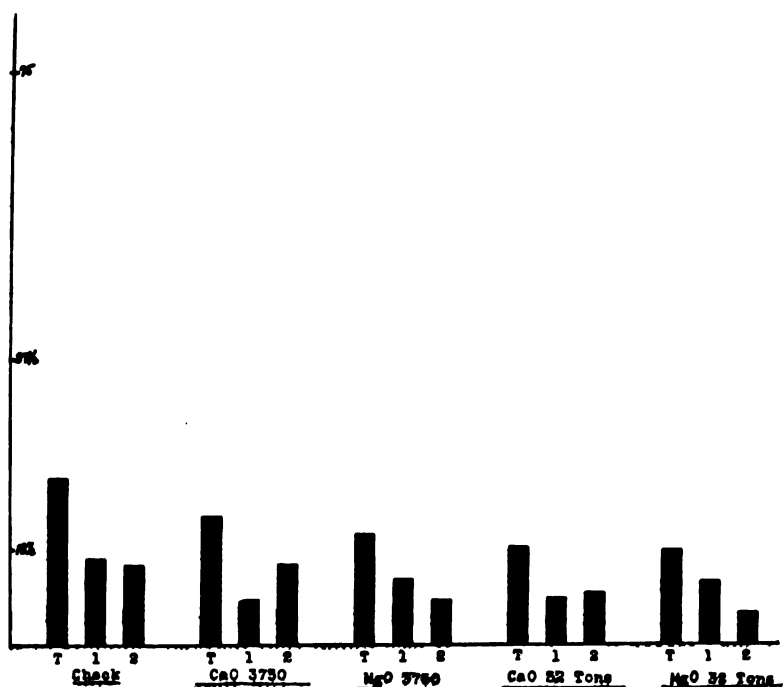
5. 18. Sulfur Treatments, Iron Pyrites = 1000 Pounds Sulfur per 2,000,000
Pounds Cherokee Sandy Loam, Surface Soil Only
T = total; numbers refer to annual periods
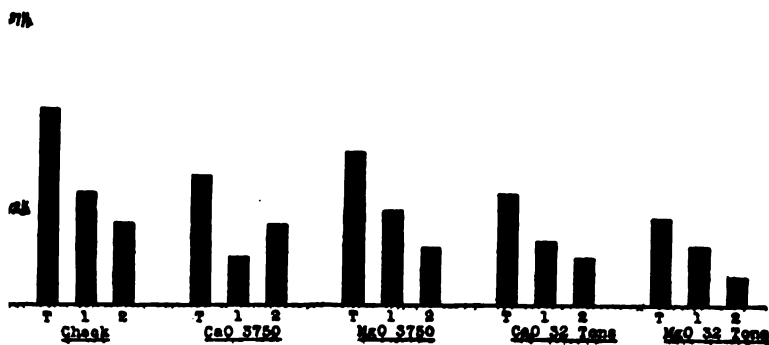
Fig. 19. Sulfur Treatments, 1000 Pounds per 2,000,000 Pounds Cherokee
Sandy Loam, Surface Soil Only
T = total; numbers refer to annual periods

data from the 22-tank lime-magnesia-sulfur series cover the initial 2-year period after installation on August 3, 1917.

First annual period. In considering first the data of table 15, we find that the average outgo of potassium from the no-sulfur lime and magnesia treated tanks amounted to 7.9 pounds as compared with 11.8 pounds for the blank during the initial year. In no case did a single light lime, magnesia, limestone, or dolomite treatment increase the leaching of potassium.

In the group of five FeSO₄ tanks, only the tank receiving the sulfate alone yielded as much potassium to the leachings as was carried through in the leachings from the blank. The FeSO₄ treatment might well be considered a treatment of H₂SO₄. However, as previously pointed out, the ferrous sulfate treatments were neutralized by the added CaO and MgO and, apparently, even by the native alkali or alkali-earth siliceous compounds of the soil. Examination of the leachings demonstrated that no free acid or FeSO₄ came through, most of the outgoing SO₄ having been in combination with calcium. This observation is interesting in its relationship to the observation of Ruprecht (28), who stated ". . . . it was found that marked amounts of soluble aluminum and iron salts were removed from the plats receiving sulfate of ammonium, by long-continued washing of the soils with distilled water . . . ." Even if all of the applied and mixed acid iron sulfate was not neutralized by the added or native alkali or alkali-earth compounds, none was yielded to the natural leachings consequent to rainfall. If the increase in potassium outgo from the FeSO₄ check, above that yielded by the blank, is considered as actually representing an increased decomposition of native soil potassic compounds, it would be difficult to determine whether the release is to be attributed to direct action of the acid radical, or to a subsequent basic exchange after the formation of CaSO₄. An actual depression in solvent action appears to have been registered in the case of both the light lime and light magnesia treatments and also the heavy lime treatment, which represents an excessive hydrated-lime treatment, plus CaSO₄.

Again, in the case of the group of five iron pyrites tanks, we find a distinct retardation in the potassium outgo for each lime and each magnesia addition. The iron pyrites check is practically identical with the blank. Sulfate determinations on the leachings from this pyrites check demonstrated that oxidation of the pyrites to FeSO₄ had occurred to an appreciable extent. With the increased SO₄ outgo, there was found a parallel increase in the outgo of calcium. Hence, this treatment might be considered as a partial treatment of CaSO₄.

In the third, or sulfur, group we find an average potassium outgo of 10.2 pounds for the initial year, as compared with 11.8 pounds for the blank. The sulfur check, however, has yielded more of potassium than has the blank; while the light application of MgO has also induced a yield somewhat greater than that derived from the untreated tank. As in the case of the pyrites check, analyses of leachings demonstrated that, in the case of both blank and

TABLE 15

Amounts of potassium leached from Cherokee sandy loam surface soil in field lysimeters—August, 1917, to August, 1918

Treatments of limestone, dolomite and oxides of calcium and magnesium, the oxides having also supplementary treatments of sulfur in the forms of ferrous sulfate, iron pyrites or elemental sulfur

TANK NUMBER	TREATMENT				TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Alkali earth		Sulfur					
	Form applied	Amount per 2,000,000 pounds of soil	Form applied	Amount of sulfur per 2,000,000 pounds of soil				
				lbs.				
50	None	None	None	None	85.7	3.11	0.2665	11.8
51	Limestone	⇔2000 lbs. CaO	None	None	85.1	2.40	0.2042	9.0
52	Dolomite	⇔2000 lbs. CaO	None	None	85.3	1.96	0.1672	7.4
53	CaO	2000 lbs.	None	None	84.6	2.46	0.2081	9.2
54	CaO	3750 lbs.	None	None	76.2	1.68	0.1280	5.6
55	MgO	⇔2000 lbs. CaO	None	None	79.3	2.21	0.1752	7.7
56	MgO	⇔3750 lbs. CaO	None	None	75.6	2.56	0.1933	8.5
	Average...				84.4	2.21	0.1793	7.9
57	None	None	FeSO₄	1000	75.2	4.45	0.3346	14.8
58	CaO	3750 lbs.	FeSO₄	1000	73.8	1.84	0.1358	6.0
59	MgO	⇔3750 lbs. CaO	FeSO₄	1000	71.5	1.69	0.1208	5.3
60	CaO	32 tons	FeSO₄	1000	76.7	1.58	0.1228	5.4
61	MgO	⇔32 tons CaO	FeSO₄	1000	54.9	4.25	0.2333	10.3
	Average...				70.4	2.76	0.1895	8.4
62	None	None	Iron pyrites	1000	83.7	3.15	0.2637	11.6
63	CaO	3750 lbs.	Iron pyrites	1000	77.3	1.92	0.1484	6.5
64	MgO	⇔3750 lbs. CaO	Iron pyrites	1000	79.7	2.44	0.1945	8.7
65	CaO	32 tons	Iron pyrites	1000	90.0	1.62	0.1458	6.4
66	MgO	⇔32 tons CaO	Iron pyrites	1000	82.1	2.26	0.1855	8.2
	Average...				82.6	2.28	0.1876	8.3
67	None	None	Sulfur	1000	81.6	4.20	0.3427	15.1
68	CaO	3750 lbs.	Sulfur	1000	73.7	2.04	0.1503	6.6
69	MgO	⇔3750 lbs. CaO	Sulfur	1000	79.4	1.74	0.2885	12.7
70	CaO	32 tons	Sulfur	1000	88.5	2.16	0.1912	8.4
71	MgO	⇔32 tons CaO	Sulfur	1000	58.3	3.10	0.1807	8.0
	Average.........				76.3	2.65	0.2307	10.2

all treatments, a considerable amount of sulfur had been converted to sulfates. In the blank, the outgo of sulfate has been accounted for principally as calcium sulfate; so that this tank represents a progressive treatment of light amounts of $CaSO_4$. Likewise, the four CaO and MgO tanks have in effect received supplementary and progressive additions of either $CaSO_4$ or $MgSO_4$.

Taken as a whole, we find that the six lime, magnesia, limestone or dolomite treatments effected a depressing influence on the outgo of potassium, where no supplementary sulfur materials were applied; that eleven of the twelve lime or magnesia treatments, with supplementary sulfur in three forms, yielded less of potassium than did the blank; and that, of the three tanks receiving the sulfur materials without lime or magnesia, one was practically identical with the blank, while the other two gave slight increase in potassium leachings. It must, therefore, be concluded that neither light nor heavy applications of burnt lime or burnt magnesia gave any evidence of liberation of native soil potassium during the initial annual period of the experiment with the Cherokee sandy loam. This accords with the initial annual-period results, obtained in the case of the Cumberland loam, where the indication of potassium liberation appeared first in the third annual period.

Second annual period. In comparing the 8.4-pound potassium yield from the blank tank no. 50 with the leachings from the limestone-, dolomite-, and oxide-treated tanks no. 51 to 56 (table 16), we find for the six treated tanks an average outgo of 6.4 pounds from consistent results, indicating that the calcic and magnesic treatments have to some extent actually depressed the outgo of potassium.

However, in the case of tank 57, receiving $FeSO_4$ alone, we find a slight tendency toward the enrichment of the potassium content of the leachings. But, where the $FeSO_4$ has been supplemented by additions of CaO or MgO in tanks 58 to 61, the depressive tendency of the oxides is again manifested.

In tanks 62 and 63, receiving respectively iron pyrites and iron pyrites plus the light application of lime, there is a small increase in the potassium outgo. The remaining three tanks, no. 64, 65 and 66 of this set, show the same tendency toward diminished potassium leachings that was noted in the case of the $FeSO_4$ group.

Considering next the sulfur tanks no. 67 to 71, we find that the continued oxidation of sulfur has resulted in considerable increase in the potassium yielded by the soil to the leachings from the tank which received sulfur alone. The light application of burnt lime effected a marked depression in the outgo of potassium salts, when compared to the check sulfur tank, although a small increase is to be noted when the comparison is made with the blank tank no. 50. A repetition in the depression of potassium outgo occurred in the case of the MgO tanks no. 69 and 71 and also in the case of the CaO tank no. 70. A consistent and marked decrease is noted in the case of tank 71 and tanks 61 and 66, corresponding treatments, with reference to MgO, though differing in the form of the added sulfur.

TABLE 16

Amounts of potassium leached from Cherokee sandy loam surface soil in field lysimeters—August, 1918, to August, 1919

Treatments of limestone, dolomite and oxides of calcium and magnesium, the oxides having also supplementary treatments of sulfur in the forms of ferrous sulfate, iron pyrites or elemental sulfur

TANK NUMBER	TREATMENT				TOTAL LITRES LEACHED	PARTS PER MILLION IN SOLUTION K	GRAMS PER TANK	POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL
	Alkali earth		Sulfur					
	Form applied	Amount per 2,000,000 pounds of soil	Form applied	Amount of sulfur per 2,000,000 pounds of soil				
				lbs.				
50	None	None	None	None	131.6	1.45	0.1908	8.4
51	Limestone	≈2000 lbs. CaO	None	None	130.5	1.21	0.1579	7.0
52	Dolomite	≈2000 lbs. CaO	None	None	130.9	1.04	0.1361	5.5
53	CaO	2000 lbs.	None	None	131.1	1.16	0.1521	6.7
54	CaO	3750 lbs.	None	None	122.3	1.27	0.1553	6.8
55	MgO	≈2000 lbs. CaO	None	None	124.4	1.11	0.1381	6.1
56	MgO	≈3750 lbs. CaO	None	None	124.9	1.15	0.1436	6.3
	Average...				127.4	1.16	0.1472	6.4
57	None	None	FeSO₄	1000	124.3	1.87	0.2324	10.2
58	CaO	3750 lbs.	FeSO₄	1000	121.7	1.36	0.1655	7.3
59	MgO	≈3750 lbs. CaO	FeSO₄	1000	118.0	1.10	0.1298	5.7
60	CaO	32 tons	FeSO₄	1000	109.4	1.10	0.1203	5.3
61	MgO	≈32 tons CaO	FeSO₄	1000	102.2	0.98	0.1002	4.4
	Average...				115.1	1.28	0.1496	6.6
62	None	None	Iron pyrites	1000	124.4	1.93	0.2401	10.6
63	CaO	3750 lbs.	Iron pyrites	1000	126.6	1.93	0.2443	10.8
64	MgO	≈3750 lbs. CaO	Iron pyrites	1000	128.2	1.06	0.1359	6.0
65	CaO	32 tons	Iron pyrites	1000	132.0	1.20	0.1584	7.0
66	MgO	≈32 tons CaO	Iron pyrites	1000	117.4	0.88	0.1033	4.6
	Average...				125.7	1.40	0.1764	7.8
67	None	None	Sulfur	1000	130.7	1.93	0.2522	11.1
68	CaO	3750 lbs.	Sulfur	1000	122.7	1.98	0.2429	10.7
69	MgO	≈3750 lbs. CaO	Sulfur	1000	123.5	1.42	0.1754	7.7
70	CaO	32 tons	Sulfur	1000	122.9	1.20	0.1475	6.5
71	MgO	≈32 tons CaO	Sulfur	1000	102.6	0.77	0.0790	3.5
	Average.........				120.5	1.46	0.1794	7.9

TABLE 17

Summary of potassium leaching data, Cherokee loam, surface soil only, for the 2-year period ending August 4, 1919

TANK NUMBER	TREATMENT				LITERS LEACHED		PARTS PER MILLION IN SOLUTION K		GRAMS PER TANK		POUNDS PER ACRE SURFACE 2,000,000 POUNDS OF SOIL	
	Alkali earth		Sulfur									
	Form applied	Amount per 2,000,000 pounds of soil	Form applied	Amount per 2,000,000 pounds of soil (lbs.)	Total	Average annual	Total	Average annual	Total	Average annual	Total	Average annual
50	None	None	None	None	217.3	108.7	4.58	2.29	0.4573	0.2287	20.2	10.1
51	Limestone	+2000 lbs. CaO	None	None	215.6	107.8	3.61	1.81	0.3621	0.1811	16.0	8.0
52	Dolomite	+2000 lbs. CaO	None	None	216.2	108.1	3.00	1.50	0.3033	0.1517	19.6	9.8
53	CaO	2000 lbs.	None	None	215.7	107.9	3.62	1.81	0.3602	0.1801	15.9	8.0
54	CaO	3750 lbs.	None	None	198.5	99.3	2.95	1.48	0.2833	0.1417	12.4	6.2
55	MgO	+2000 lbs. CaO	None	None	203.7	101.9	3.32	1.66	0.3133	0.1567	13.8	6.9
56	MgO	+3750 lbs. CaO	None	None	200.5	100.3	3.71	1.86	0.3369	0.1685	14.8	7.4
Average					208.4	104.2	3.37	1.69	0.3265	0.1633	15.4	7.7
57	None		FeSO4	1000	199.5	99.8	6.32	3.16	0.5670	0.2835	25.0	12.5
58	CaO	3750 lbs.	FeSO4	1000	195.5	97.8	3.20	1.60	0.3013	0.1507	13.3	6.7
59	MgO	+3750 lbs. CaO	FeSO4	1000	189.5	94.8	2.79	1.40	0.2506	0.1253	11.0	5.5
60	CaO	32 tons	FeSO4	1000	186.1	93.1	2.68	1.34	0.2431	0.1216	10.7	5.4
61	MgO	+32 tons CaO	FeSO4	1000	157.1	78.6	5.23	2.62	0.3335	0.1668	14.7	7.4
Average					185.5	92.8	4.04	2.02	0.3391	0.1695	14.9	7.5

62	None.........	None	Iron pyrites	1000	208.1	104.1	5.08	2.54	0.5038	0.2519	22.2	11.1
63	CaO.........	3750 lbs.	Iron pyrites	1000	203.9	102.0	3.85	1.93	0.3927	0.1964	17.3	8.7
64	MgO.........	⇐3750 lbs.CaO	Iron pyrites	1000	207.9	104.0	3.50	1.75	0.3304	0.1652	14.7	7.4
65	CaO.........	32 tons	Iron pyrites	1000	222.0	111.0	2.82	1.41	0.3042	0.1521	13.4	6.7
66	MgO.........	⇔32 tons CaO	Iron pyrites	1000	189.5	94.8	3.14	1.57	0.2888	0.1444	12.8	6.4
	Average......				206.3	103.2	3.68	1.84	0.3640	0.1820	16.1	8.1
67	None.........	None	Sulfur	1000	212.3	106.2	6.13	3.07	0.5949	0.2975	26.2	13.1
68	CaO.........	3750 lbs.	Sulfur	1000	196.4	98.2	4.02	2.01	0.3932	0.1966	17.3	8.7
69	MgO.........	⇐3750 lbs. CaO	Sulfur	1000	202.9	101.5	3.16	1.58	0.4639	0.2320	20.4	10.2
70	CaO.........	32 tons	Sulfur	1000	211.4	105.7	3.36	1.68	0.3387	0.1694	14.9	7.5
71	MgO.........	⇔32 tons CaO	Sulfur	1000	160.9	80.5	3.87	1.94	0.2597	0.1299	11.5	5.8
	Average......				196.8	98.4	4.11	2.06	0.4121	0.2051	18.1	9.1

Aggregate of potassium leached during 2-year period. When we compare the respective averages of the limestone, dolomite, CaO or MgO treatments for the 2-year period (table 17) we find that in no one case, of the 18 tanks which received calcic or magnesic materials, has there been indicated any interchange of calcium or magnesium for the native soil potassium. Rather, there has been indicated for the 2-year period a distinct diminution in the amount of potassium leached, where additions of the alkali-earthy materials have been made.

As noted in the discussion of the 5-year period results from the Cumberland loam soil, there has ensued as a result of both lime and magnesia treatments, either a depression of potassium liberation, or else an enhancement in the retentive properties of the soils for any potassium possibly liberated. Following the line of reasoning mentioned previously in quoting the work of Shreiner and Failyer (30), it would appear that had such liberation of potassium resulted from treatments of lime and magnesia, any immediate tendency to reabsorb the liberated potassium would be mitigated by the ultimate tendency toward attainment of equilibrium between the absorbed potassium-enriched surface film and the free water of the soil. Hence, as in the case of the preceding studies with the Cumberland loam soil, the Cherokee sandy loam results indicate that when either lime or magnesia is added to a long-leached residual soil there occurs an actual depression in the concentration of potassium salt in the free soil water, rather than a liberation of potassium, with consequent enhanced supplies of potassic salts available to the roots of growing plants. However, it may again be pointed out that an extensive plant root development might be expected to secure potassium compounds more readily from any liberated and reabsorbed potassium than from the potassium compounds in their natural condition. Nevertheless, assuming an interchange between calcium or magnesium and native soil potassium, and assuming wheat to be a representative crop, the results of Gaither (12) and those of MacIntire (19) previously cited, would indicate no such activity on the part of the plant, where every opportunity was afforded for such assumed potassium liberation. The same obtains in the case of the plant-ash results for corn, oats, wheat, and grass, as reported by Lyon and Bizzell (18).

PRACTICAL VALUE OF RESULTS

In summarizing the 5-year-period results from the Cumberland loam and the 2-year-period results from the Cherokee sandy loam, in their application to and interpretation into practice, it would seem that one definite and positive conclusion is justified, with reference to these two types of soil under the prevailing climatic conditions, to wit: *practical or economical applications of burnt calcareous limestone, burnt dolomitic limestone, ground calcareous limestone, or ground dolomitic limestone will not effect a direct chemical liberation of native soil potassium.*

Some indication of potassium liberation was obtained where CaSO₄ was engendered from sulfureous materials incorporated with the Cherokee sandy loam, in the absence of supplementary lime treatments. However, with this particular soil, the meagerness of the indicated liberation, as compared with the intensity of treatment, was such as to minimize the dependence to be placed upon economical amounts of calcium sulfate fertilizers as liberants of soil potassium in amounts sufficient to be of practical value. This, however, is without prejudice with reference to the need for, or value of, the sulfur content of such materials.

However, the probable fixation of nitrogen; improved tilth, particularly through increase in the amounts of more active organic matter; enhanced biological activation and other cumulative benefits resulting from the judicious use of lime or limestone may be expected to effectuate such a marked general improvement in the plant feeding zone as to accelerate the hydrolysis of potassic silicates and diffusion of soluble potassium salts to the roots of the growing plants. Such secondary benefits might be fully equivalent, in their liberative action, to that which would be possible were chemical substitution of calcium for potassium actually accomplished.

SUMMARY AND CONCLUSIONS

It is pointed out that deductions from laboratory studies have led to the assumption that lime may be considered as a liberant of native soil potassium, to the extent of benefiting plant growth.

Review of the literature indicates that such an assumption is not justified in the case of the forms of lime used in practice, with the possible exception of calcium sulfate.

Analytical data from the Pennsylvania station demonstrated that, after long usage, burnt lime and ground limestone caused a marked depression in the potassium content of wheat straw.

The obscurely recorded analytical results obtained upon the K₂O analysis of the limed plats of the Pennsylvania station are quoted *in toto*. The fact is pointed out, that the error of sampling such large areas is a factor to be considered, and that a comparison based on the results from the upper two 7-inch zones supports different conclusions from those that would be drawn from a consideration of the three 7-inch zones.

The Cumberland loam lysimeter experiments, embracing 46 tanks, with and without subsoil, and receiving varying amounts of nine different forms of lime and magnesia are considered with reference to the total potassium leached during each of five annual periods.

During the initial annual period, the deep tanks gave a greater average outgo of potassium than did the shallow, surface-soil tanks. The subsoil tanks were more uniform in their yield of potassium than were those of the surface soil. No increase of potassium outgo occured coincident with increase in the amount of treatment.

The same observations hold for the results of the second year. During the third and very humid annual period, the heavier applications of burnt lime began to show a marked effect in the liberation of potassium from the surface soil. The potassium liberated to the free soil-water was absorbed by the subsoil. The averages of the results induced by the other treatments show a marked uniformity within each series, as well as a close parallel between the outgo from the surface-soil tanks (excluding the 32-ton and 100-ton CaO treatments) and that from those containing both surface soil and subsoil. The individual and average amounts passing from all treatments were considerably less than those obtained from the no-treatment tanks.

The amounts of potassium leached during the relatively dry fourth annual period were distinctly smaller than were those yielded during the preceding year, the subsoil tanks again giving somewhat higher results. The 32-ton CaO surface-soil tank returned to normal, coincidently with the disappearance of Ca(OH)₂ from the soil. The 100-ton burnt lime surface-soil tank still yielded an excessive outgo of potassium, as compared with the other oxide and carbonate treatments. Both surface-soil and surface-soil-subsoil tank blanks again yielded more potassium to the leachings than did any single treatment (other than CaO, 100 tons), or the average of treatments.

The fifth annual period registered the liberative action of the 100-ton CaO treatment and the subsequent absorption of the liberated potassium by the subsoil. Again, each calcic and magnesic treatment at each rate (excepting the 100-ton CaO treatment) has depressed the potassium content of leachings, as compared with the blanks of both surface-soil and surface-soil-subsoil tanks.

The summation of the data of the 5-year period indicates that the magnesic compounds exert a somewhat more depressive influence on the outgo of potassium salts, than do the corresponding lime compounds, though no great differences are manifest. Excepting, after the second year, tanks 8 and 15, which still contained residual Ca(OH)₂ from heavy applications of burnt lime, all of the calcic and magnesic treatments have yielded smaller amounts of potassium to the leachings than were yielded by the no-treatment tanks. The extent of outgo of potassium appears to have been uninfluenced by the intensity or magnitude of the applications of carbonate forms. Excepting the burnt lime tanks, the several treatments show a relatively close concordance within each of the two systems of different depths. However, the subsoil tanks demonstrated closer concordance, and an average outgo greater than that from the surface tanks.

It is pointed out that CO_2 solutions in H_2O are apparently more active upon native soil potassic complexes than is the same hypothetical acid, when it is in part neutralized by the formation of acid carbonates of calcium or magnesium.

When considered in connection with the cited plat results and plant-ash analyses, the data of the 46-tank series demonstrate that it is not tenable to assume that native soil potassium is liberated from the silicate forms to the free soil-water by ordinary applications of the oxides, or the several carbonates of cal-

cium or magnesium, in light or heavy amounts. Nor does it appear probable that potassium is released from siliceous combination and furnished to the film water, there to be concentrated and held by physical absorption against the tendency to diffuse to the more dilute free soil-water. Rather, it would appear that, were liming to induce any enhancement in the amounts of potassium elaborated by the plants, such an effect would be the result of accelerated plant growth, thereby causing greater range and more vigorous root action and root decay, which are calculated to extract more potassium from the native stores of potassium, unaltered by any lime or magnesia treatments in reasonable amounts.

Thirty-two-ton and 100-ton treatments only have given any indication of potassium liberation; and such liberated potassium has, in time, diffused to the natural leachings. This tends to emphasize the fact that, in all other cases, the diminished potassium leachings were actually due to a lesser solvent action of the free soil-water, when it became impregnated with calcium or magnesium bicarbonates. For, had the other forms effected any potassium liberation, dynamic tendencies would have resulted in an ultimate enrichment of the potassium content of their free-water solutions.

Reference is made to the plats of the Tennessee station which show that burnt lime, in 1-ton applications, has failed to rectify the definite need of potassium manifested in crops of cowpeas and wheat, in a rotation composed exclusively of these two crops.

The 22 lysimeters containing Cherokee sandy loam afford an opportunity to study the effect of single light applications of burnt lime, burnt magnesia, limestone and dolomite upon the outgo of potassium. They also furnish opportunity for observations upon the potassium-liberating tendencies of calcium and magnesium sulfate engendered from $FeSO_4$, iron pyrites, or sulfur, when these three materials are used alone and together with light and heavy applications of CaO or MgO.

During the initial annual period, each unsupplemented form of lime and magnesia depressed the outgo of potassium, while each lime and magnesia treatment supplemented by $FeSO_4$, iron pyrites or sulfur produced the same effect, with one exception.

The results of the second annual period demonstrate a continued depressive effect on potassium outgo as a result of single treatments of lime, magnesia, limestone, or dolomite. The same tendency is manifested in 10 of the 15 treatments where CaO and MgO singly were supplemented by $CaSO_4$ or $MgSO_4$, formed or engendered within the soil, from the treatments of $FeSO_4$, iron pyrites or sulfur. The distinct depressive tendency of heavy applications of MgO, even when augmented by engendered $MgSO_4$, was particularly consistent.

The average of the two year's potassium outgo from the Cherokee soil shows a distinct depressive action induced by CaO, MgO or limestone when applied separately and alone; and the same result likewise for lime and magnesia when used in connection with $FeSO_4$, iron pyrites or sulfur. The three sulfur compounds when used alone gave, in each case, a slightly greater yield

of potassium; but, it is not clear whether this was due to direct action of the engendered acid, or to substitutive action of the calcium and magnesium sulfates formed therefrom within the soil.

Again, as in the case of the Cumberland loam, even the 32-ton application of burnt lime failed to register any increase in the potassium yielded to the outgoing free soil-water during the first two years after treatment.

The results from these two residual soils demonstrate that the original free soil-water leachings are richer in potassium salts than they are when they become impregnated, in slight or excessive degree, with calcium or magnesium bicarbonate derived from any one of the several forms of these two alkali-earths. Furthermore, excepting, in the case of the Cumberland loam, the excessive and impractical 32-ton and 100-ton CaO treatments, nothing that has been developed would suggest the possibility that the lime, magnesia or their several chemical or mineral carbonate treatments have so altered the original form of soil potassium as to render any residual portion of it more available or beneficial to plant growth.

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PLATE 1

Fig. 1. General View of Hillside System Used for Exposure and Leaching Experiments

Fig. 2. Inside View of Hillside System Used for Exposure and Leaching Experiments

FIG. 1

FIG. 2

LUENCE OF SODIUM CHLORIDE UPON THE PHYSIOLOGICAL CHANGES OF LIVING TREES

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.rious observations in the neighborhood of factories, salt refineries and nines seem to have led to the general belief that sodium chloride always ι harmful effect on plants and trees. Our knowledge of the rôle of chlo- and sodium in plant growth is very limited. Only recently an attempt been made to study more fully the influence of sodium chloride upon : growth.

REVIEW OF LITERATURE

ιe literature on the subject of fertilization with common salt presents .derable evidence that often an increase in yield may be expected, but a ber of investigators report failures with the use of salt for certain agri- ιral crops. Agriculturists in different countries have been using sodium ·ide to a certain extent, but the beneficial effects were often ascribed to sodium-ion only, due perhaps to the view that sodium might replace ssium. In recent years, however, several investigators take the view- t that the chlorine-ion might have a direct fertilizing value, or an indirect izing value by the acceleration of certain processes. An indirect result ιt be caused by the increase of acidity in certain soils, which would make .in plant-food constituents more available.

Effect on exterior form

udies made by a number of investigators of the effect of the physiological ges on the exterior form of plants, caused by applications of sodium chlo- often show marked responses. Dassonville (8) observed that the mor- ɔgical characters of *Lupinus albus* under the influence of different salts greatly modified. Hansteen (12) found that wheat plants grown in ιral solutions containing chlorides produced larger roots and shorter :s than when grown in solutions containing other salts. Marked changes ιe leaf structure and the transpiring power of wheat have been observed [arter (14). Saline soils containing 1, 1.5 and 2 per cent of total salts, hich 0.7, 1 and 1.4 per cent were NaCl (70 per cent), were diluted with en loam on the basis of dry weight. The mixed soils were then placed

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in containers sealed with paraffine to make comparative transpiration meas-
urements by weighing possible. Plants of wheat, oats and barley grown
from seeds in these saline soils very soon developed a pronounced waxy bloom
on the leaf surface and a thickening of the cuticule occurred. The thickness
of the cuticule increased with the concentration of the soil. The size of the
epidermal cells decreased as the concentration of the salt in the soil increased,
the cells of the plants grown on the non-saline soils being the largest. Leaves
of wheat detached from plants grown in non-saline soils on which the bloom
was not conspicuous, lose by transpiration two to three times as much mois-
ture as leaves from plants grown in soil containing 1 per cent of sodium chlo-
ride. Nobbe and Siegert (24, 25) reported a higher transpiration in buck-
wheat plants grown in water cultures containing NaCl. Investigations made
by Laurent (20) with sugar beets and carrots in connection with the plant-
food supply in its relation and variation in plants, showed that NaCl in soil
had the effect of lengthening both carrots and beets, but reduced the diameter
of these plant roots. Buckwheat grown to maturity in water cultures by
Tottingham (32) showed that the length of roots and the production of dry
matter in leaf blades was depressed in solutions containing an addition of
NaCl. Sugar beets when grown in the greenhouse were more watery where
chlorides were applied, and the yield of the dry matter was greatly increased.
Van Hecke (17) conducting experiments with salt to prevent the gummosis
of fruit trees, treated the trees with salt, giving 1, 2 and 3 pounds per tree,
and found at the end of the season that the tree receiving 3 pounds did not
suffer from gummosis, while the ones receiving 1 and 2 pounds were slightly
injured by the decrease. The other untreated trees were severely injured,
lost most of their branches and bore but little fruit. The effect of salt water
on cultivated plants is reported by Wohltmann (35). If the amount of salt
was from 5 to 10 gm. per liter of solution the growth of the better grasses and
leguminous plants was dwarfed and the yield reduced. Common salt used
as top-dressing on oats in the manurial experiments carried on by the Agri-
cultural Society of Scotland (38), gave satisfactory results against lodging,
but the effect of top-dressing with salt on barley reported by the Board of
Agricultural Education in Great Britain (36, 37) was to cause the barley to
lodge rather than to stiffen the straw.   ·

Effect on chemical composition

Physiological changes influencing the chemical composition of plants have
been reported in a great number of cases. Hartwell and Wessels (16) found
that the per cent of phosphorus in crops treated with salt as a fertilizer was
increased. Studying the influence of chlorine compounds in crude Stassfurt
salts upon the composition of potatoes, Sjollema (28) found that the effect of
the chlorine salts was to lower the starch content. Süchting (31) came to
the same conclusions, while Tottingham (32) found that by an application of

sodium chloride alone the composition of the potato tubers was only slightly altered, but the quality was seriously affected. According to Bolin (5, 6) the dry matter per hectare is not less with NaCl. De Ruijter de Wildt et al (9) found that although the yield of beets was high with an application of 300 kgm. NaCl per hectare, the salt content of the soil had changed their composition, reducing the sugar content, changed the relation of potassium and sodium by greatly increasing the sodium content and also increased the content of chlorine and of ash. Micheels (23) studying the effect on germination, length of leaf, length of root and weight of wheat plantlets grown in solutions of NaCl and KCl, reports that the chlorine-ion was harmful, decreasing the lengths of root-hairs and of leaf, and depressing the weight of the plants. Nobbe (25), giving data on ash analyses for buckwheat, points out that the per cent of ash in roots was increased by sodium chloride. Comparing garden plants with his culture plants the increase was from 6.8 to 15.3 per cent for roots and from 8.7 to 18.6 per cent for stems. Prinsen Geerlings (27) who made a study of the influence of sodium salts in the soil upon the composition of sugar cane, found that an application of either NaCl, $CaCl_2$ or $MgCl_2$ to the soil caused an increase in potash in the ash of the cane.

Toxic effects of sodium chloride

It seems that a number of investigators hold the view that sodium chloride, even in small amounts, is not only harmful but also dangerous to plant life. Still Hugo de Vries (33) pointed out already in an early study that it is "a wrong viewpoint however widely held, that strong salt solutions (for example a sodium chloride solution of 10 to 20 per cent) are dangerous for the life of plant cells." Harter (15), who made extensive comparisons of his own results with those of other investigators relative to the growth of wheat seedlings in salt solutions, concluded that sodium chloride in a concentration of about 300 parts per million, is not toxic to these plants. Guthrie and Helms (11), working with pot cultures to determine the limits of endurance of different farm crops to injurious substances, found that a salt solution of 0.05 per cent prevented germination of wheat by only 0.2 per cent, of corn by 0.25 per cent and of rye by 0.2 per cent. The growth was affected to a still lesser degree. Hendry (18) using 13 varieties of legumes in pure quartz sand with NaCl of 0.04, 0.16, 0.3, 0.5 and 1 per cent of the dry weight of the sand, found that as a general rule the germination was retarded, the height of the plants was lessened, the blossoming period changed, a reduction in number and size of the leaves was caused, and finally premature death resulted. Birger (3), however, studying the influence of sea-water upon the germination of seeds, placed 27 species of Scandinavian seeds for a period of 30 days in sea-water containing 3.4 per cent of salt, and found that although the vitality of a number of species was destroyed, the vitality of others was little or not at all affected. In some instances he found that the number of seeds which ger-

minated was increased by having been in the sea-water for 30 days as compared with dry seeds. By the extensive investigations on the effects of chlorides in alkali soils of Utah, Harris (13) found sodium chloride the most toxic of a number of common chlorides. He found that at a concentration of 0.2 per cent sodium chloride in the soil, the germination of wheat was reduced by 50 per cent.

EXPERIMENTS

In a cut-over swampy soil on the College Farm at New Brunswick, N. J., a number of selected tree stumps, which has been allowed to grow for 5, 6 or more years, were treated with common rock salt. One hundred and thirty stumps of as nearly equal size as could be found were selected, including white oak, black oak, birch, maple, and a few chestnut, dogwood and

TABLE 1

Chosen tree stumps treated with common rock salt in 1919

NUMBERS	APPROXIMATE AVERAGE HEIGHT	SALT APPLIED PER TREE	VARIETY OF TREE			
			Oak	Birch	Maple	Chestnut, etc.
	feet	*pounds*				
1 to 10	11	1	2	2	5	1
11 to 20	12	2	3	2	2	3
21 to 30	13	3	4	2	1	3
31 to 40	13	4	7	2		1
41 to 50	13½	5	3	4	3	
51 to 60	12	6	7	1	1	1
61 to 70	13½	7	7	1	2	
71 to 80	13	8	6	1	2	1
81 to 90	14	9	8	1		1
91 to 100	14	10	5	1	3	1
101 to 130	14	None	14	6	5	5
Total..........................			66	23	24	17

cherry. The trees were treated in the manner given in table 1. The stumps were surrounded by a large number of tree stumps, which could also be used to collaborate the results. Only thirty trees not treated with salt, however, were under constant observation.

Analyses made of the rock salt used, show from 96 to 98 per cent sodium chloride, very little magnesium chloride and some impurities.

The salt was applied in a dry state, on top of and around the stumps. The application was made during the days of April 3, 4 and 5, 1919. The weather was sunny and dry, but soon after the application heavy rains partly dissolved the salt. It was not until far along in the summer that all of the salt was dissolved where the heavier applications were made.

A general examination on May 14 showed that some birch trees which were given the greater amount of salt (above no. 60) were already injured, the

TABLE 2

Condition of tree stumps four months after the treating with common rock salt

NUMBER OF TREE STUMP	VARIETY	APPROXI-MATE HEIGHT	SALT APPLIED	CONDITION AUGUST 7, 1919
		feet	*pounds*	
1	Birch	12	1	Healthy appearance
2	Oak	9	1	Healthy appearance
3	Maple	12	1	Slight yellowish tint in most of the leaves
4	Maple	7	1	Healthy appearance
5	Maple	15	1	Vigorous growth, leaves large
6	Maple	8	1	Leaves yellowish tinted
7	Maple	8	1	Healthy appearance
8	Birch	15	1	Healthy
9	Oak	12	1	Healthy
10	Dogwood	10	1	Healthy
11	Dogwood	10	2	Healthy
12	Oak	12	2	Healthy
13	Birch	18	2	Vigorous growth; leaves dark green
14	Chestnut	12	2	Injured; leaves curling, yellowish
15	Chestnut	11	2	Healthy; vigorous growth
16	Maple	10	2	Healthy
17	Birch	20	2	Vigorous growth; leaves extremely large and shiny; dark
18	Oak	16	2	Vigorous growth
19	Maple	6	2	Seriously injured; leaves curling; brown at the edges
20	Oak	15	2	Healthy; broad, shiny leaves
21	Oak	10	3	Healthy; bluish-green, large leaves
22	Oak	10	3	Bluish colored, large leaves
23	Oak	11	3	Healthy; bluish-green, large leaves
24	Chestnut	7	3	Injured; all leaves dropped except leaves of top shoots
25	Maple	8	3	Injured; leaves at the top of the branches dead; leaves only one third of normal size
26	Birch	13	3	Slightly injured; leaves brown at the tips and somewhat at the edges
27	Birch	18	3	Healthy
28	Cherry	17	3	Healthy
29	Walnut	10	3	Healthy
30	Oak	8	3	Healthy; leaves shiny; bluish color
31	Birch	18	4	Healthy; thrifty
32	Oak	15	4	Slightly injured; leaves brown at the edges and slight change in tint of color towards yellow
33	Oak	8	4	Injured; leaves brown, curled
34	Cherry	10	4	Injured; leaves curled; sickly appearance
35	Oak	12	4	Thrifty; large leaves
36	Oak	15	4	Main stem healthy; side stems injured; leaves curling with brown edges
37	Oak	13	4	Healthy
38	Oak	9	4	Healthy; head veins of leaves have a slightly yellowish tint

TABLE 2—*Continued*

NUMBER OF TREE STUMP	VARIETY	APPROXI-MATE HEIGHT	SALT APPLIED	CONDITION AUGUST 7, 1919
		feet	*pounds*	
39	Oak	17	4	Healthy; bluish-green leaves; thrifty
40	Birch	18	4	Sickly appearance; leaves large, yellowish in color; catkins have a peculiar conical shape (plate 4)
41	Oak	12	5	Healthy
42	Birch	15	5	Older leaves small and sickly; second-growth shoots are large and shiny; catkins conical shape
43	Birch	15	5	Top shoots dead; leaves brown and curling; catkins deficient in vigor
44	Birch	12	5	Healthy; head veins of leaves slightly yellow; no catkins
45	Birch	18	5	Healthy; catkins conical
46	Maple	13	5	Old leaves small and sickly in appearance; new shoots with thrifty large leaves
47	Oak	18	5	Healthy
48	Maple	14	5	Leaves partly curled as if there was lack of moisture; sickly appearance
49	Oak	13	5	Healthy
50	Maple	8	5	Leaves which are left curled; sick appearance; partly dead
51	Oak	12	6	Healthy
52	Birch	14	6	Dead; small new shoots died also
53	Oak	10	6	Partly injured; main stem thrifty; injured leaves brownish and curling
54	Sumach	10	6	Sickly appearance; leaves drooping
55	Oak	18	6	Healthy; second growth shoots have large leaves
56	Oak	10	6	Healthy, with large leaves
57	Oak	12	6	Healthy
58	Oak	12	6	Healthy
59	Maple	8	6	Dead
60	Oak	15	6	Healthy with bluish-green, shiny leaves
61	Oak	18	7	Healthy with bluish-green but small leaves
62	Oak	12	7	Injured; leaves brown and curling
63	Oak	13	7	Healthy
64	Oak	18	7	Leaves large; bluish in color, shiny; vigorous growth in new top shoots; leaves of tall main stem (18 feet) much larger than leaves of side stems (8 to 9 feet), which are slightly injured leaves of side stems spotted with small yellow blotches; part of leaves curling
65	Oak	15	7	Healthy; veins on the back of the leaves a little yellow
66	Birch	12	7	Dead
67	Oak	15	7	Partly injured; leaves brownish in color
68	Oak	12	7	Partly injured; leaves on main stem healthy

TABLE 2—*Continued*

NUMBER OF TREE STUMP	VARIETY	APPROXI-MATE HEIGHT	SALT APPLIED	CONDITION AUGUST 7, 1919
		feet	*pounds*	
69	Maple	8	7	Injured; leaves dropped; making second growth but leaves tiny and curled, clustering around the stem; size of new leaves from one-tenth to three-tenths of normal size; these small clustered leaves wither after a short time
70	Maple	10	7	Seriously injured; only a few leaves (top) of highest branches left, these brown and curled
71	Oak	10	8	Injured; all leaves brownish; lower leaves of branches dropping, remainder had a sick appearance; curling
72	Birch	13	8	Dead
73	Oak	14	8	Slightly injured; leaves brownish in color
74	Oak	17	8	Partly injured; leaves with brown edges
75	Oak	13	8	Injured; leaves yellow; sickly appearance; curling
76	Oak	15	8	Veins on the back of the leaves yellow; developing later into spotted leaves
77	Cherry	13	8	Injured; leaves yellowish
78	Oak	18	8	A number of leaves brown, dried; some with only brown edges
79	Maple	10	8	All leaves dropped and very small new shoots formed which dried out later
80	Maple	10	8	All stems dead, but a few small new shoots starting from roots
81	Oak	12	9	Slightly injured; second growth extremely vigorous
82	Chestnut	18	9	Seriously injured; all leaves brown and curling
83	Oak	13	9	Injured; sickly appearance. (A birch standing nearby killed by the same dose)
84	Oak	12	9	Partly injured; most of the leaves look sickly, with a shiny brown surface
85	Oak	11	9	Injured; brownish leaves, curling at the edges
86	Oak	16	9	Seriously injured; highest leaves of top shoots dropped and remainder brown and sickly
87	Oak	16	9	Yellow tint on all leaves
88	Oak	15	9	Injured; leaves brownish with a rubber-like consistency
89	Birch	15	9	Seriously injured; only few leaves at the end of the highest shoots left. (A maple standing nearby also badly injured by the same dose)
90	Oak	18	9	Partly injured; leaves of the shorter stems brown and curled; those of the higher stems, however, very large
91	Oak	18	10	All veins on the back of the leaves yellowish, giving the appearance of lace-work

TABLE 2—*Concluded*

NUMBER OF TREE STUMP	VARIETY	APPROXI-MATE HEIGHT	SALT APPLIED	CONDITION AUGUST 7, 191?
		feet	*pounds*	
92	Oak	12	10	Injured; sickly appearance; leaves of local discolorations, starting ? end of the small veins and grac ing; after some time leaves bec have a rubber-like consistenc? break or tear these leaves
93	Oak	12	10	Part of the leaves brown and curle
94	Maple	8	10	All leaves that are left are brown a
95	Birch	15	10	Most of stems dead; a few seriousl one apparently healthy; smal from roots .
96	Maple	12	10	Dead
97	Ash	10	10	Dead, except two small new shoots the roots
98	Oak	17	10	Slightly injured; only few leave brownish
99	Maple	12	10	No leaves of the first growth left, ? shoots of the second growth ap? small shoots dried out
100	Oak	15	10	Sickly appearance like no. 92; only branches left; all leaves near st giving the stems the appearanc the late autumn, when a few l? left before heavy frost
101–130	Oaks, Maples, Birches, Chestnuts, etc.		None None None None	All trees healthy throughout the except two of the chestnut tree? sick; their leaves being brownis? toward the end of the season

young leaves having a withered appearance. The salt was at th yet completely dissolved under these injured trees. On June ? trees which showed injury at the earlier date were making new sp before the second growth of the control birch trees started. T oaks appeared at this time more thrifty than the untreated, havi darker green foliage. An examination on June 15 showed that ? and birch trees which received the larger application were dying the oaks with the same application were injured; the leaves get edges and slightly curling. Those with smaller amounts had an e ferent appearance. Here the salt seemed to act as a fertilizer broad, heavy, dark bluish-green leaves.

An individual examination was made on August 7, at the he? season. The results are given in table 2.

DISCUSSION

The physiological influence of sodium chloride upon the different species of trees is by no means the same. The susceptibility to injury differs greatly not only for the different species, but also within the same species. Individuals, often standing close to each other, and apparently growing under the same circumstances—as far as moisture, weather conditions and soil type go—react so differently that it is difficult to say where the injury of a certain amount of salt starts and where the stimulating or beneficial effect stops. This is especially true for the hardy oak woods. The soft-wood trees are much more easily injured. However, the range of injury for maple and birch is also rather wide. A small application of salt acts apparently as a fertilizer for oak trees. Some trees make even a vigorous growth with an application of 7 pounds of sodium chloride. In some cases injury occurs when 4 pounds are applied. In the case of birch trees slight injury is recorded by an application of 3 pounds. The range for birch trees, of a height of approximately 15 to 18 feet, between stimulation and injury seems to be approximately 2 to 3 pounds. The maple, which grows fast and consequently takes up moisture more rapidly for transpiration of food materials, etc., is affected by as little as 1 or 2 pounds of salt.

As a rule the stimulating or fertilizing effect results in longer shoots and larger leaves which have often a darker green color than the leaves of untreated trees. The surface of these leaves is shiny, sometimes having a waxy consistency. The larger the leaves are, the darker green is their color, and the more glossy is their surface. With oak trees the leaves are not only extremely large, but also a greater number have developed. This gives the leaves the effect of a very luxuriant, tropical growth.

The injury by all trees is shown in a somewhat similar way. In several cases the main (tallest) stems of oak trees were affected in such a way, that the leaves were large, bluish-green, with a vigorous growth in the secondary top shoots, while leaves of the side (shorter) stems showed slight injury. In several cases the leaves of the different stems of the same tree were measured. Number 64 for instance (plate 1) shows a number of leaves of the main stem which had an average length (measured from base to tip) of 30 cm. and a width of 24 cm., while the average length of a number of leaves of the side stems was only 15 cm. with a width of 12 cm. All leaves were taken from top shoots. The shape of the leaves, due to their great difference of dimensions, was accordingly different, the deep incisions more or less disappearing with increasing size. These large leaves were quite often bloated, the parenchyma bulging up between the primary and secondary veins, giving the leaves an unnatural, dropsical appearance (plate 1, fig. 1). Signs of injury of the oak trees started usually at the extreme end of the smaller veins. At the end of the tracheids occurred a very slight discoloration, probably due to the chlorine-ion entering the cell and changing the composition of the chlorophyll.

This slight discoloration extended until the leaf was spotted with yellow-brown regions. These regions touched each other after some time and gave the leaf a peculiar sickly appearance. If the injury showed at the extreme edges first, the leaf would turn brown at those places and curl; the center of the leaf might remain green for some time afterward, until the injury proceeded, resulting finally in brown closely curled leaves. In the former case the leaves may keep their usual flat surface until they dry. In both cases the leaves quite often become very glossy, dry out and become rubber-like. It is very difficult to break or tear these leaves. Sometimes the injury appears at first only in the primary and secondary veins, usually best seen on the under-side of the leaves. A light yellow discoloration becomes evident, giving the leaves an appearance of being temporarily covered with a fine yellow lace-work. The badly injured oak trees do not always shed their leaves, but as far as could be observed, the shedding took place in all cases where the leaves did not curl. Curling of the leaves as a rule appeared first on the lower branches, the leaves closest to the stem being attacked first. The injury spread gradually by attacking the higher leaves, and the leaves of the top shoots were affected last. This resulted in several cases in giving the trees in midsummer an appearance of autumn trees before heavy frost has removed the leaves of the end branches and top shoots.

Although the influence of sodium chloride on the birch trees was similar to that on the oaks, in some respects the results were different. Trees which were given smaller applications made vigorous growth, the leaves of the treated trees becoming larger and more glossy than those of the untreated trees. Instead of having a greater number of leaves, however, as in the case of oaks, the shoots quite often were elongated so much that the leaves seemed to be placed farther apart no. 40, plate 3). Where only 1 or 2 pounds of the salt was applied, the trees were very luxuriant but when given 3, 4 or 5 pounds injurious effects were predominant. The leaves became slightly yellow-spotted and turned more or less suddenly into the typical autumn yellow color of birch trees. After dropping these leaves, tiny new shoots with light yellowish-colored leaves appeared, which stood usually close to the stems. These shoots were probably the latent buds forced to grow under the influence of the sodium chloride in an effort to survive. These small new shoots would soon turn black and dry out (plate 3). In some cases a third effort was made by the trees which sent out small new shoots from the roots. In nearly all cases these young stems suffered the same fate, as the tiny new "secondary" shoots.

Another peculiarity is shown in plates 3 and 4. The trees which produced the elongated branches looking as though they were outgrowing themselves, made a peculiar-shaped catkin. The catkins while in bloom were observed to have an abundance of stamens on the part nearest to the stems, while the upper part was relatively poor in stamens. This resulted in giving the catkins a conical, pointed shape. The writer was not able to detect an untreated

birch tree in this swamp which had similar-shaped catkins. They occurred only when the tree was moderately injured. Photographs (plate 4) of the dry catkins made in the middle of November show the same rather pronounced, pointed shape, due to fewer and smaller seeds. The seeds of the tips of these catkins were smaller, the wings of the seeds toward the upper ends compared with the wings of the nutlets nearest to the stem were much smaller, the length and the width of the wings gradually decreasing toward the tip of the catkin. The salt seemed to shrivel these catkins in a similar way as it did the leaves. Whether the shriveling is due to the chlorine itself or to the preventing of the necessary food materials from entering the cells, could not be made out.

The soft-wooded maples were affected most quickly. Even when an application of only 1 pound of sodium chloride was made, a yellowish tint occurred in the leaves. This yellow tint changed rapidly into a black-brown by a heavier application. The leaves curled, became brown at the edges and finally dropped. The leaves at the end of the top shoots were quite regularly injured first, the other leaves of these branches following and the leaves of the lower branches, progressing in the same order, last. The brown edges were very dry, and broke easily by slight rubbing or when the wind shook the branches. If the wind moved the branches more vigorously, the leaves rattled as leaves will do which have remained on the trees in the fall. The new shoots of the secondary growth in the cases of slight and moderate injury were very short. They would cluster together, curling, and had a yellow-green color. The leaves and branches which survived never became larger than from one-tenth to three-tenths of the ordinary size of leaves (plate 6). The older injured leaves began gradually to drop soon after the heavier applications were given, and the tree made the usual effort to survive by sending out small new shoots from latent buds (plate 6). Those tiny branches clustered around the stem, became yellow, curled and dropped in nearly all cases.

If the height of the trees was taken into consideration it was quite evident that the injury decreased with the increasing size of the trees. A same application by weight would often do no harm, or injure only slightly a tall tree, while a lower one of the same species would be badly injured or sometimes killed.

It does not seem justifiable to describe in detail and draw conclusions from the behavior of the chestnut trees. Only comparatively few chestnuts were included and two of the five labelled "control" had a sickly appearance at the height of the season. Some of the same kind of trees standing in the swamp were also brownish and sickly. The data of the few cherry and dogwood trees in this experiment are given only for completeness of the record. All twenty-five trees marked "control" which were under constant observation, besides hundreds of others in the swamp were healthy throughout the entire season.

POSSIBLE EFFECTS OF CHLORINE

Whether it is a combination of the sodium and chlorine-ions or the chlorine-ion alone which causes the physiological responses within the cell, is not known. Bolin (4) concludes from his various fertilizer tests with a number of different soils, that the influence of the NaCl used, depended on its chlorine content, but not on its content of sodium. Söderbaum (29), experimenting with a poor sandy soil deficient in chlorine, admits that the remarkable increase might have been caused partly by the replacing of potassium by sodium, but the proportionate growth was beyond the amount of potassium and phosphorus added. He used three portions of soil, adding a complete fertilizer containing nitrogen in the form of $NaNO_3$, $(NH_4)_2SO_4$ or NH_4Cl. Sodium chloride was added in equivalent amounts to the $NaNO_3$. A noticeable increase in yield in behalf of the salt when added to the $NaNO_3$ and $(NH_4)_2SO_4$ was found, but not with NH_4Cl. There could not have been any hygroscopic action of the salt, as the pots were constantly watered. The investigator estimates an absorbed amount of 0.126 gm. of chlorine by the plants. These investigations were repeated and correlated by Söderbaum (30) in 1915. He makes the statement (page 20): "It can hardly be doubted any longer, the chlorine is an important factor in physiological changes."

Only hypotheses are given by some of the investigators concerning the way sodium chloride may react within the plant cell. The fact that there is a relation between the chlorine content and the accumulation of starch in potatoes, led Pfeffer and his students (26) to the suggestion that enzyme action might be the controlling factor. Tottingham (32) makes the supposition that the concentration of chlorine in the cell may exert marked controlling influences upon the activities of intracellular enzymes and if, in turn, many vital activities of the plant are controlled by enzyme action, the physiological responses might be brought about. From the data secured in our experiment it seems quite evident that the injurious and stimulating effects are caused mostly by the chlorine. Whether the chlorine combines with the chlorophyll or acts upon it in some other way to destroy it, can not be made out as yet. It is possible that the chlorine increases the acidity in the cell, which could accelerate the vital activities or enzymatic actions to a certain extent. By a too large increase of acidity the acceleration would change into a toxic action. The fact that applications of small amounts of NaCl which resulted in a luxuriant growth, giving the leaves an extremely dark color, change to a toxic effect whereby the leaves become gradually brown, when a greater dose is given, seems to support this supposition. Another observation seems to point in the same direction. A comparison between dead and live wood of trees of the same species, showed that the hydrogen-ion concentration differs. The pH values for live wood were lower than for dead wood, indicating that the acidity of these live trees was higher than of the dead trees. The preliminary studies on this part of the subject, however, are not yet far enough advanced to permit of drawing conclusions.

SUMMARY

Experiments were made upon 100 trees (oak, birch and maple) with sodium chloride; applications ranging from 1 to 10 pounds were made to individual trees.

Some trees showed injury as early as 6 weeks after the application, while after 10 weeks a number of trees were seriously injured and some dying. The leaves of these trees turned brown and curled. An examination made at the height of the season showed some marked external changes. Smaller applications of salt apparently acted as a fertilizer. The trees treated with a small application were making a vigorous growth, the leaves becoming very large and thick, having a dark blue-green color and glossy surface. Others elongated their branches making the distance between the leaves unusually wide. The first signs of toxicity appeared usually at the edges of the leaves at the extreme end of the tracheids, or in the primary and secondary veins. The injury spread gradually until the leaves had a spotted sickly appearance. After some time the leaves dried out with a rubber-like consistency. These leaves kept their flat and glossy surface and dropped from the branches. If the injury started at the edges of the leaves, they gradually turned brown, curled, but remained on the trees. When the injury appeared in the veins first a beautiful yellow-colored lace-work seemed to cover the under-side of the leaves.

Quite frequently the trees made an effort to survive by sending forth tiny new branches from latent buds. These small branches in nearly all cases turned black-brown and dried out.

Most of the trees which were given smaller applications made a secondary growth long before the untreated trees standing nearby.

Of the trees experimented with, the maple is the most easily affected by sodium chloride, followed by the birch and finally by the oaks.

The rate of injury seems to be dependent upon the height of the trees. The higher trees were more resistant than the lower ones of the same species.

It is possible that the chlorine increases the acidity in the plant cell, accelerating or harming the vital activities, according to the amount employed.

The fact that a small amount of sodium chloride acts as a fertilizer or as a stimulant for trees and shrubs, may lead to the more general use of common rock salt for certain plants, while the toxic effects of the larger applications might be employed in the eradication of weeds and the clearing of farm land from live stumps.

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PLATE 1

FIG. 1. Two top branches of the same oak tree showing the difference in length and widr ████th of leaves. The shape of the leaves is changed, because of the great difference in dimension████ ███. The largest leaves show unnatural, vigorous growth, with the parenchyma bulging up betwe████████ the primary and secondary veins.

FIG. 2. Another view of the smaller branch in figure 1, showing signs of injury starti██████ing with the lowest leaves of side branches and top shoots; leaves beginning to curl.

FIG. 1

FIG. 2

PLATE 2

FIG. 1. Oak branch with leaves which have a sickly appearance. Injury starts at the extreme end of the tracheids and proceeds. Leaves do not curl.

FIG. 2. Oak branch showing injury which results in the dropping of the leaves; this gives the tree an autumn-like appearance.

PLATE 2

FIG. 2

FIG. 1

PLATE 3

Fɪɢ. 1. Birch with elongated shoots as result of the application of NaCl.   Catkins have a conical shape.

Fɪɢ. 2. Birch with shoots from latent buds, which turn black-brown, dry out and drop off.   Leaves from last year's buds have dropped previously.

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FIG. 2

FIG. 1

417
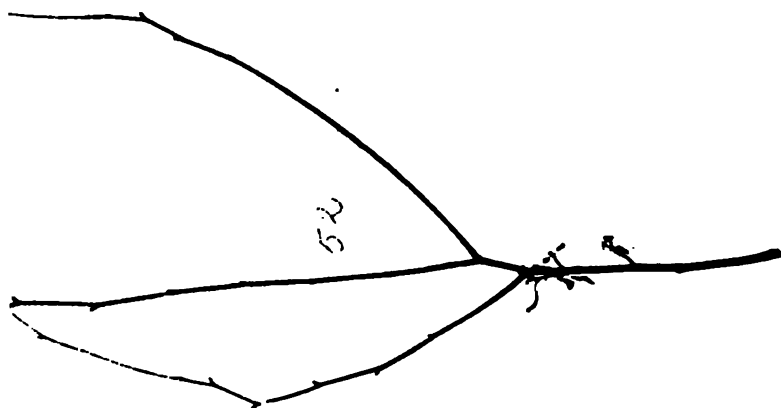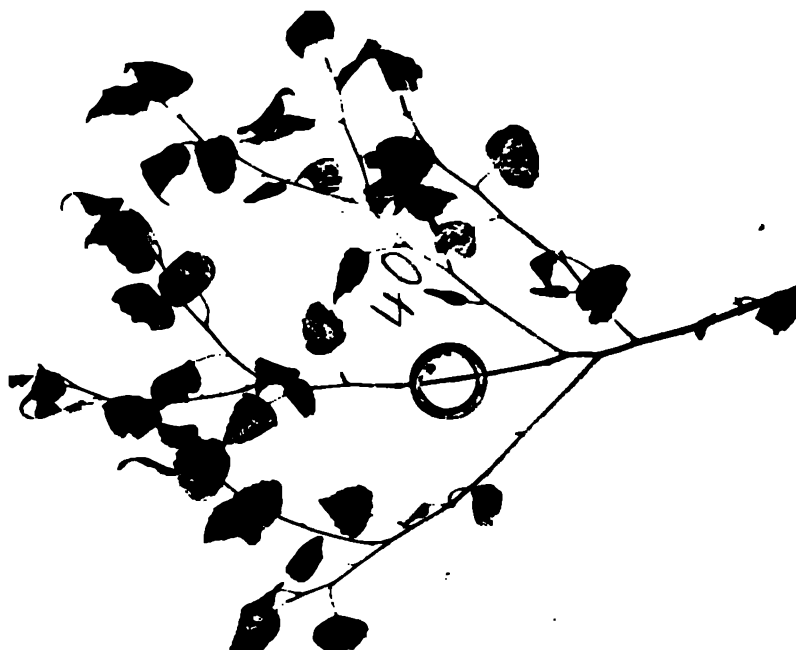
PLATE 4

Fig. 1. Catkins on branches of birch trees treated and untreated with NaCl; the former having a conical, pointed shape.

Fig. 2. Catkins removed from branches; about two-thirds actual size. Conical catkins have fewer and smaller nutlets toward the tips.
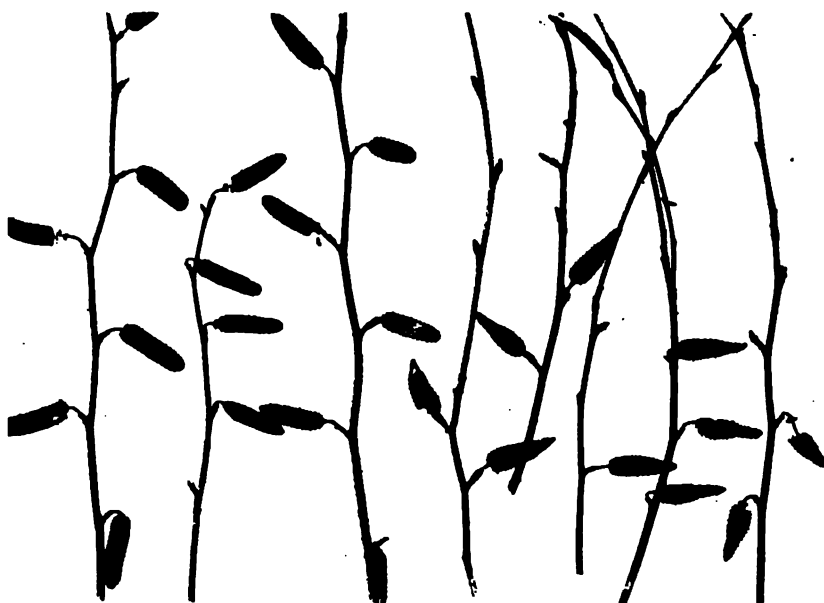
FIG. 1 (Upper)   FIG. 2 (Lower)

PLATE 5

FIG. 1. Branch of treated chestnut tree.

FIG. 2. Branch of maple showing beginning of injury on the end leaves of the shoots. The highest leaves are affected first.

FIG. 2

FIG. 1

PLATE 6

FIG. 1. Injured maple at a further stage than in plate 5. These leaves dry out and bre———k by slight rubbing or by swaying in the wind.

FIG. 2. Tiny shoots from latent buds made in an effort to survive. These leaves whi⸺ch never grew larger than one-tenth to three-tenths of the usual size, became yellow a———nd curled, and dropped off.
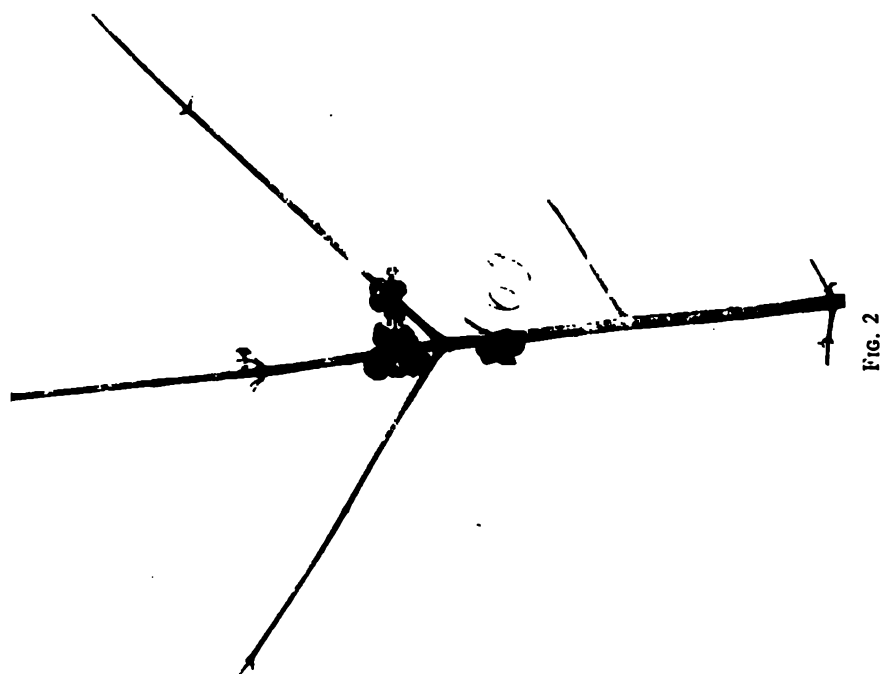
FIG. 2

FIG. 1

PLATE 7

FIG. 1. Treated birch dying on August 7.

FIG. 2. Treated oak with autumn-like appearance. All leaves nearest to the main st̲e̲m have dropped.

FIG. 3. Maple which made large leaves in secondary growth. Older leaves all dropped. Only the new leaves on the top shoots remained.

W. RUDOLFS
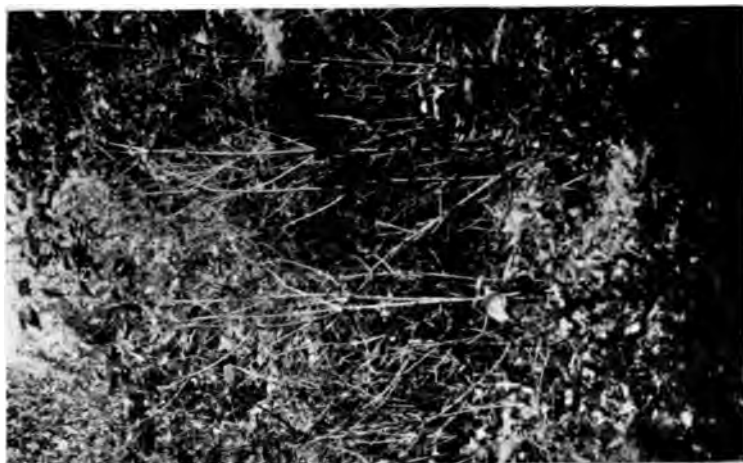
FIG. 3

FIG. 2

FIG. 1

THE ISOLATION AND STUDY OF NITRIFYING BACTERIA[1]

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INTRODUCTION

The formation of nitrates from organic nitrogenous substances in soil has been known for a long time. As far back as the middle of the nineteenth century the nature of this process was discussed and was considered by the majority of investigators to be purely chemical. This chemical view was held by Kuhlman (30), Dumas (17), Mulder (36) and other investigators, and was further substantiated by the great chemist Liebig (32) whose powerful influence no doubt prevented an earlier discovery of the actual cause of nitrification. The results of Boussingault (12) (1860) demonstrated that the nitrogen of nitrates does not come from the supply of the air. In 1878 Schlöessing and Müntz (51), from a study of sewage disposal, presented a most remarkable paper in which they demonstrated that the oxidation of ammonia to nitrite is due to microörganisms found in soil. Their results led to numerous attempts to isolate the organism, but without success. Warington (59, 60, 61), Frank (18), Frankland (19), Adametz (1), and Heräus (24) attempted to secure the organisms in pure culture but were unsuccessful.

HISTORICAL REVIEW

Warington (59, 60, 61) began the study of nitrification prior to 1878, and continued it through to 1890. Although his cultures contained a large number of ammonia-oxidizing organisms, he was not able to isolate on agar or gelatin-plates pure cultures of this organism. He secured many pure cultures from plates but never the true ammonia oxidizer. Similar attempts to secure pure cultures of the nitrite-oxidizing organism resulted in failure.

The Franklands (19) (1890) worked on the problem of the isolation of the nitrite-forming organism about the same time as Warington. In 1890 they published a report in which they state that all contaminating forms were eliminated from their cultures, except one, which would grow in bouillon but would not oxidize ammonia. By repeated dilution experiments they claim to have

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eliminated this last form and thus to have secured a pure culture of the nitrite-forming organism.

Jordan and Richards (27) (1888–1890), observed the oxidation of ammonia to nitrite and nitrate in samples of natural waters. Upon investigation they found that the nitrifying organisms were invariably present in Boston tap-water. Attempts to isolate the organism by the gelatin plate method resulted in failure. Many organisms were isolated but none would oxidize ammonia in pure culture or with a mixed culture of strains isolated from the original water. Dilution methods were employed and an organism obtained which oxidized ammonia to nitrite and subsequently to nitrate. Jordan and Richards are the first American investigators to report the isolation of nitrifying bacteria.   .

The classical nitrification experiments of Winogradsky (63, 64, 65) beginning in 1890, stand out preeminently, and have never been repeated. The results of his earlier work, namely, the discovery that organic matter in culture media is toxic to sulfur bacteria, no doubt proved very helpful in his investigation of the nitrifying bacteria. Winogradsky was able to isolate the nitrifying organisms by use of a medium containing strictly pure inorganic salts. First he carried his mixed cultures through many enrichments, i.e., several transfers in selective media. He then inoculated gelatin plates from these enrichment cultures and secured the ammonia-oxidizing organism (*Nitrosomonas*) in pure culture by the negative plate method, i.e., from portions of the plate which showed no visible growth. Later silicic acid gel plates were prepared, containing ammonium sulfate and other inorganic salts, and the colony characteristics of the organism studied in pure culture. Washed agar was used with similar success. One year later he repeated the work and isolated the nitrite oxidizer (*Nitrobacter*). Winogradsky states that both nitrifying organisms are slow to develop on solid media and are visible first under the microscope after an incubation period of 2 to 6 weeks. Growth on the plates is detected first by chemical tests. After all of the ammonium sulfate or the sodium nitrite is oxidized more is added in small wells made by removing portions of the medium, and in this manner the size of the colonies increased. He describes the nitrite-forming organism (*Nitrosomonas*) as an oval or ellipsoidal cell 0.9 to 1 μ wide and 1.2 to 1.8 μ in length. There are two types, one persisting in zooglea-like growth, the other in single cells; the former produces no turbidity in the solution, while the latter produces turbidity. The single-celled form, termed "Schwärmer" is motile, with a single polar flagellum. The colonies on silicic acid gel appear (enlargement of 100 diameters) as small refractive bodies with sharp outline, at first colorless, later brown and finally a characteristic dark brown. Some colonies remain compact while others have a main central kernel surrounded by single cells. The colonies are exceedingly tenacious and difficult to tear apart with the platinum needle. The nitrate-forming organism (*Nitrobacter*) is 0.3 to 0.4 μ wide and about 1 μ long, the cells occurring singly or in pairs and occasionally in threes. They

ire spindle-shaped, non-motile, and possess a capsule which makes them dif-
icult to stain. After a period of 10 days colonies on washed agar appear as
,trongly refractive bodies at a magnification of 150 to 200 diameters, and after
.wo to three weeks are rounded, oval, or heart-shaped, 30 to 50 μ in diameter,
omewhat brown and shiny. Winogradsky isolated these organisms from the
,oils of many different countries and found that they varied only slightly in
norphology.

He carried out many experiments and found in all cases that soluble organic
natter is very toxic to these organisms. Moreover, in coöperation with
)meliansky (67), he noted that glucose, peptone, asparagin, glycerin, and urea
ire fatal in quantities of 0.2 to 1 per cent. Bouillon was used as a test for
)urity, and if no growth was visible at the end of 10 days the culture was con-
iidered pure. They found that the carbon must be derived from free carbon
lioxide or the bicarbonates; carbon from organic substances or from normal
:arbonates is not available. For each part of carbon assimilated by *Nitro-
iomonas* 35 parts of nitrogen are oxidized to nitrite, and for each part assim-
lated by *Nitrobacter* 40 parts of nitrite nitrogen are oxidized to nitrate.

From 1892 to 1896, Godlewski (21, 22, 23) worked with nitrifying organisms
ind by means of a series of carefully planned experiments confirmed the work
)f Winogradsky in regard to their source of carbon. He later repeated the
work in mixed cultures and found that nitrification will not take place unless
:arbon dioxide is present.

In 1895 Burri and Stutzer (13, 14) claim to have isolated the nitrate-forming
)rganism, and stated that it grew in bouillon. After a controversy of consid-
:rable duration, Winogradsky (65) secured one of their supposedly pure cul-
tures and found that it contained other organisms. He isolated from this
:ulture the true nitrate-forming organism and two other forms. Two years
later Stutzer and Hartleb (56) took up the problem again, made enrichment
:ultures, and inoculated agar plates. After a period of 4 to 6 weeks the nitrite
in the plates was oxidized and colonies developed which converted nitrite to
nitrate in liquid media but would produce no growth in bouillon. Agar
ilopes containing nitrite were inoculated and after a long period of incubation
growth developed which was not continuous over the agar but made up of
many very small beads. When examined in the hanging drop, the organisms
were found to have yeast-like buds. They also isolated *Nitrosomonas* by use
)f magnesium-ammonium-phosphate ($MgNH_4PO_4$) agar plates. On this
phosphate medium colonies developed after 4 weeks; but no growth could be
iecured on silicic acid gel. Stutzer claims to have isolated the organisms
from the gel by the negative method.

In 1897 Stutzer and Hartleb (56) attempted to explain the presence of the
contaminating forms found in their culture by Winogradsky, and did further
work on the carbon relation of the organism in pure culture. Four years later
they published a remarkable paper (57) concerning the isolation of the nitrify-
ing organisms. By the use of enrichment cultures and washed agar they isolated

a nitrate-forming organism to which they gave the name *Nitromicrobium*. Their description of the physiological properties of the organism is similar to that given by Winogradsky for *Nitrobacter*, and in morphology it varied but little from *Nitrobacter*, the chief difference being a yeast-like budding of *Nitromicrobium*. They reported also the presence of another organism which was strikingly similar to *Nitrobacter* and *Nitromicrobium* but did not oxidize nitrite to nitrate. This non-nitrifying form they called *Hyphomicrobium*. The colony characteristics were much the same as those of *Nitrobacter*. In common with *Nitrobacter*, a medium containing nitrite was essential for the growth of *Hyphomicrobium*. This organism was oval in shape, 0.8 μ wide and 1.2 to 1.5 μ long. It would not grow in bouillon but would grow in a solution containing asparagin or ammonium sulfate. The greatest difference between *Hyphomicrobium* and *Nitrobacter* was a thread-like growth of very small rods which could be seen growing from one pole, or sometimes both poles, of the *Hyphomicrobium* cells. The authors are firm in their statement that Winogradsky did not at all times have a pure culture because he used the bouillon test as a criterion of purity and there are other organisms which will not grow in bouillon, for example *Hyphomicrobium*. This report contains 21 photomicrographs of the organisms. Stutzer (55) later reported on the isolation of *Nitrobacter* by means of unwashed agar plates. The bud-like growth was again observed. Magnesium-ammonium-phosphate agar plates were used successfully for the isolation of *Nitrosomonas*. Silicic acid gel was also used but did not give satisfactory results. The nitrite-forming organism was secured from the gel by the negative method.

In the literature on nitrification it seems that this report of Stutzer and Hartleb has been overlooked. The writer of the paper presented herewith has noticed a very short stem-like growth in many stains of his pure cultures of *Nitrobacter*. Such growth is very noticeable when the preparation is stained by any method for staining flagella, but is seldom seen in the ordinary stained preparation. Results obtained by the writer lead him to believe that these investigators were nearer the truth than is commonly thought.

Beddies (5, 6), in 1899, reported the isolation of nitrifying organisms. He found that the organisms were not very sensitive to high concentrations of organic matter and were aided by the presence of compost and humus in the medium. The solid medium he used consisted of silicic acid gel containing 1 per cent of a concentrated humus solution. The organisms were able to form spores under certain conditions.

In 1899, Omeliansky (40–44) isolated the nitrite-forming organism and introduced gypsum blocks as a suitable solid substratum. He prepared them by adding 1 per cent magnesium carbonate ($MgCO_3$) to calcium sulfate ($CaSO_4.H_2O$) and mixing with sufficient water to make a dough, which was then spread out on a smooth surface and allowed to dry. When hard the blocks were placed in petri dishes, sterilized, and the solution containing the desired salts poured into the dish. The nutrient solution readily diffused through

the solid gypsum block, the surface of which was inoculated with the desired culture. He reported a yellow, wart-like growth on the surface of the block. This method has the advantage that the old solution may be removed and fresh solution added; it has the disadvantage that observation under the microscope is difficult and unsatisfactory. In later reports (45, 46) Omeliansky used filter paper pads instead of gypsum. He found *Nitrosomonas* was easily stained with carbol fuchsin, but that *Nitrobacter* was much more difficult to stain.

Boullanger and Massol (11) (1903) claim to have isolated both of the nitrifying organisms. They state that silicic acid gel was used as the solid medium but do not give any cultural characteristics or purity tests. They found the thermal death point of *Nitrosomonas* to be 45°C., and that of *Nitrobacter* 55°C. A concentration of 30 gm. of ammonium sulfate in 1 liter greatly retarded nitrite formation and 20 gm. of magnesium nitrite ($MgNO_2$) retarded nitrate formation.

Fremlin (20) (1903) reported on the isolation of *Nitrosomonas*. He secured enrichment cultures and inoculated plates of silicic acid gel, agar and gelatin. Growth developed on all the plates. He claims to have obtained a pure culture. He reported that *Nitrosomonas* would grow in organic media. This conclusion was further substantiated by inoculating a slope of beef-broth agar from the pure culture, and securing a moderate growth. A nutrient ammonium sulfate solution was inoculated from the slope, and the solution then filtered several times through sterile soil under aseptic conditions. The ammonia was oxidized to nitrite while a control solution filtered through soil in similar manner produced no oxidation.

Berstyn (8) (1903) describes with exceptional thoroughness the forms which contaminate *Nitrobacter* enrichment cultures. He found the following species in the F_{20} generation of *Nitrobacter* cultures,—*Bacterium comes, Bacterium modestum, Bacterium debile,* and *Pseudomonas humicola.* He states that these forms are easily satisfied in food requirement and develop in bouillon or gelatin in very dilute solution, but will not remain alive in distilled water. They develop in the inorganic nitrifying solution unless the salts used have been re-crystallized and the water distilled from permanganate and sulfuric acid. If this precaution is taken the forms will be eliminated in the enrichment process. A very insignificant amount of organic matter is necessary to meet their requirement.

Wimmer (62) (1904) claims to have isolated the organisms, although his methods are not given. He states that at first neither *Nitrosomonas* nor *Nitrobacter* grew in bouillon, but in later experiments with the same cultures *Nitrobacter* grew. From tests with the cultures made in sand, the author concludes that organic matter is not so toxic as in solution, but is nevertheless toxic in great concentration.

Perotti (48, 49) (1906) experimented with the nitrite-forming organism. These were found in groups of 2 to 6 collected on the magnesium carbonate in

the bottom of the flask, but no zooglea forms were noted. He considered it the same as the organism described by Winogradsky, the "Westeuropaischen" form.

Bazarewski (4) (1906) isolated pure cultures of the nitrate-forming organism. Experiments were carried out in sand, the results of which confirmed other reports concerning the toxicity of soluble organic compounds. On the other hand, organic matter in soil was found not toxic. The most favorable temperature in pure culture was 37°C. In soil and sand cultures 1 per cent of dextrose stimulated nitrate formation while larger amounts delayed the process but did not entirely prevent it. From his results the author concluded that denitrification and nitrification go hand in hand in soil.

Coleman (15) (1908) worked with the nitrifying organisms and performed experiments in sand and soil. He found that 1 per cent of dextrose was not toxic, but that 2 per cent was extremely toxic. Tests for purity were made with bouillon and Heyden Nährstoff agar. The culture of *Nitrosomonas* was reported impure. The ratio of nitrogen to carbon for *Nitrobacter* was found to be about 40 to 1.

Owen (47) (1908) reported the isolation of pure cultures of the nitrifying organisms and experiments with pure cultures. The organisms were used in the study of "The Effects of Carbonates on Nitrification." Very little detail of the work of isolation is reported. He states that plates were made on washed agar and pure cultures secured from the colonies which developed. The agar gave better results than silicic acid gel. No specific tests for purity were made. Photomicrographs of the organisms show vast differences in size and shape of the individual cells.

Makrinoff (34) (1909) isolated *Nitrosomonas* and *Nitrobacter* and carried out experiments to test the effect of organic matter on pure cultures. He prepared gypsum plates (10 gm. $CaSO_4.H_2O$) containing from 0.25 to 6.5 gm. of soil each, and after the addition of the nutrient solutions inoculated them with pure cultures of the organisms. The soil did not prove toxic but caused a darker color in the organisms on the plates containing the higher amounts. Quantities of soil from 0.5 to 12.5 gm. were placed in 20-cc. portions of liquid medium, and then sterilized. The cultures receiving the 12.5 gm. of soil required 20 days for nitrite formation, while those receiving the smaller amounts of soil required only 5 days. Soil extract stronger than 2 per cent inhibited oxidation. However, a solution containing 2 per cent of soil extract was not toxic when used with plates of gypsum. The report contains photomicrographs of the plates, showing the growth of the organisms, which in general was abundant. This is an important paper on the subject of nitrification.

Beijerinck (7) (1914), reported on the isolation and cultivation of *Nitrobacter* on silicic acid gel and agar plates. He found the organism much the same in every respect as that described by Winogradsky, except in its relation to bouillon. His culture of *Nitrobacter* grew in bouillon; but by so doing it lost its power to oxidize and did not regain this power of oxidation even after

the lapse of 10 years. The oxidizing form he termed *Nitrobacter oligotrophic* and the non-oxidizing form in bouillon he termed *Nitrobacter polytrophic*. The crude cultures contained several contaminating forms which were very difficult to separate from the nitrate-forming organism. Several of these forms belonged to the family of *Actinomycetes*. The chief forms found in the nitrifying cultures he termed *Actinobacilli*. A small bacillus was also described which formed a dark brown or red pigment and was similar in gross appearance to the nitrate-forming organism. The red pigment was identified as carotin. *Bacillus nitroxus* was also present and proved the most difficult of all to separate from *Nitrobacter*. The development of unusually large colonies in pure culture on agar indicated the transformation from the active to the inactive stage; the large colonies never again produced oxidation when transferred to suitable media. He found that less than 1 per cent of sugar, mannite, sodium or calcium acetate, peptone, tyrosin, or asparagin caused the loss of oxidizing power. Colonies were evident on washed agar within 2 days. The fact is strongly emphasized that pure cultures do not grow so rapidly as mixed cultures.

Joshi (28) (1915) reported a new nitrite-forming organism which showed decided pleomorphism. This organism was commonly found in two forms, one of which was chalky white in appearance, thread-like, and long and branching like a mold, while the other form was shorter and had flagella at one pole. It would not grow in bouillon or on gelatin, and preferred magnesium carbonate as a base. The thermal death-point was found to be between 70 and 80°C. His work has not been confirmed by any other investigator.

Allen and Bonazzi (2) (1915) began the enrichment process preliminary to the isolation of the organisms. The *Nitrosomonas* cultures were carried to the F_4 generation but were abandoned at that point because of their low oxidizing efficiency. *Nitrobacter* was carried to the F_{18} generation with success. Neither organism was isolated. Bonazzi (9) in 1919 published a report which deals with the conditions favorable for nitrite formation. He found that a slow rotary movement of the culture greatly increases oxidation.

In his first experiment he states that the solution was inoculated with a pure culture. In experiments immediately following, solutions were inoculated with cultures nearly pure, while in later experiments the solutions were inoculated without reference to purity. It is apparent throughout the work that the author has met with difficulty in experimentation with pure cultures. He later reported (10) on the isolation of the nitrite ferment and gave a brief description of the organism in pure culture. There can be no doubt that the author actually obtained a pure culture of *Nitrosomonas*. This latter report is very brief and the methods employed are inadequately described. The tendency of the organism to lose its oxidizing power as a result of cultivation in liquid medium is admitted, and the necessity for special apparatus to insure aeration emphasized.

Hopkins and Whiting (25) (1916) reported on the isolation of *Nitrosomonas*. This report is indeed unusual. The details of isolation are not given; but experiments with pure cultures are discussed. In liquid cultures colonies formed on the surface in a bluish mass, some developing to $\frac{1}{4}$ inch in diameter. Silicic acid gel, inoculated from impure soil infusion, developed colonies of *Nitrosomonas* $\frac{1}{8}$ inch in diameter. They were colorless to opalescent at first and later a glassy blue, with the center showing yellow after 14 days and later orange-yellow to brown. "Visible growth in solution was slow for the first 40 days; but after that time a very profuse surface growth developed showing large blue colonies some of which were drawn up the sides of the flask by the surface tension of the liquid, and there developed to a large size ($\frac{1}{4}$ inch in diameter)." They state that *Nitrosomonas* rendered rock phosphate soluble in artificial medium and therefore acted similarly in the field. This conclusion provoked considerable criticism. The writer has made repeated attempts to confirm this work of isolation, but has never been successful. No other investigator has recorded the development of such large colonies even after extensive investigation; in fact the persistently small colony is a confirmed characteristic of both nitrifying organisms.

Russel and Bartow (50) (1916) reported work with pure cultures of *Nitrosomonas* and *Nitrobacter* isolated from activated sludge. Washed agar and silicic acid gel prepared after the method of Stephens and Temple (53) were used in isolation. Both types of media afforded visible growth after an incubation of 9 to 11 days, but no growth could be secured on gypsum blocks. The organisms would not appreciably oxidize the sludge after they had been isolated, but required the addition of a portion of fresh sludge or mixed cultures of organisms isolated from the sludge. The authors consider this proof that the nitrifying bacteria require the aid of other forms to oxidize the sludge in a sewage disposal plant. However, they state that *Nitrosomonas* and *Nitrobacter* are responsible for the nitrification of the sludge. Very little proof for the purity of their cultures is presented.

Müntz (37, 38) (1890) reported on the occurrence of nitrifying organisms. They were found in great numbers at the summit of high rocky cliffs, where nothing was present but bare rock, from which he concludes that the organisms are important in the decomposition of rock. The period of their development in this particular place was very short because of the relatively short summer. He also reported their presence in ice-clad material which had been in that condition for many years, the organisms remaining alive but inactive.

Jensen (26) (1899) studied the presence of the nitrifying organisms in the soils of Denmark. His determinations consisted of inoculating ammonium sulfate nutrient solutions with small amounts of the various soils. In peat and highland soils alike, he reported the presence of very small numbers of these organisms. Experiments with old soils of various types also indicated the presence of nitrifying organisms in very small numbers.

Thomsen (58) (1907) reported the presence of nitrifying bacteria in the sea. He found the organisms in the water at all depths up to 100 meters and often at even greater depths. However, they were found only in water adjacent the shore which indicated that the organisms were recently carried out by the water currents.

Karpinski and Niklewski (29) (1908) experimented with the nitrifying organisms in crude cultures, "Rohkulturen," and confirmed the conclusions of other investigators in regard to the toxic action of soluble organic matter. Niklewski (39) later made extensive experiments to determine the presence of these organisms in manure and urine. From his experiments he concluded that they were present in fresh manure and urine but always from outside contamination. They were found in numbers as high as 10,000 per gram in the outer layers of relatively fresh manure, while the deep manure contained only a very small number, probably because of insufficient aeration. Manure extract and urea exerted a toxic action on these organisms. The organisms found were considered to be the same as those isolated by Winogradsky.

Millard (35) (1911) reported on the number of nitrifying organisms found in soil as determined by the selective culture method. He observed a maximum number of 100,000 nitrifiers per gram of dry soil. No nitrifying bacteria were found in cow dung.

Leone and Magnanimi (31) (1891) reported on the spontaneous nitrification of gelatin in solution. After 45 days at 32°C. the nitrogen was converted to nitrate. They found more nitrogen at the end than at the beginning of the experiment, indicating the chemical fixation of nitrogen from the air.

Loew (33) (1891) discussed the following equations for the oxidation of ammonia to nitrite in the nitrification process:

(1) $2 NH_3 + 3O_2 \rightarrow 2 NO_2H + 2H_2O$ (not true)
(2) $2 NH_3 + 2O_2 \rightarrow 2 NO_2H + 4H$ (true)
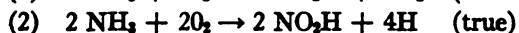
He holds that equation (1) is not true but that equation (2) represents true conditions. The hydrogen is used to reduce the carbon dioxide as follows:

$$CO_2 + 4H \rightarrow CH_2O + H_2O$$
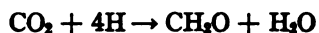
The formaldehyde (HCHO) is not condensed to a carbohydrate but is directly synthesized to protein.

PURPOSE OF INVESTIGATION

This is a report of investigations planned to study primarily the relations of the nitrifying bacteria to their environment. It is reasonable to believe, if these organisms can be isolated and can be brought to function in pure culture, that some of their relations may be explained. If they cannot be made to function normally in pure culture, then the result is equally important. Nitrification will not take place in a solution which is acid, yet some acid soils

support nitrification even better than soils which are neutral or alkaline in reaction. Whether or not this is true with pure cultures has never been determined. It is possible that other organisms present, which are favored by the acid condition of the soil, make possible the growth of the nitrifying organisms. If such a symbiotic relation exists, it can be determined only by the use of pure cultures. The fact that organic matter is not toxic in soil when mixed cultures are used does not signify that such matter is not toxic to pure cultures. The breaking down of this material through the action of other organisms is generally accepted as the explanation of the fact that soil organic matter is not toxic; yet this fact has never been established by experimental evidence. Work with pure cultures might determine whether or not this hypothesis is correct.

In addition to the many questions which present themselves, the problem of securing pure cultures is a large one in itself. The methods of isolation have been improved but little since the appearance of Winogradsky's first paper on this subject. The relationship which exists between these organisms and their related forms is still a mystery. The bouillon test for purity has given conflicting results and we do not know whether or not this can be used as a test for purity. This paper deals with the work of isolation and morphology of the organisms, a presentation of methods employed and results secured. It is hoped at a later date to publish a more complete report of the physiology of these organisms in pure culture.

EXPERIMENTAL DATA

Methods

The methods and media used are primarily those offered by Winogradsky and Omelianski in their first work on isolation, combined with certain modifications. A few new methods will be outlined, some possibly not practical for other lines of work but primarily adapted to the isolation of the nitrifying organisms.

Liquid media. All solutions were prepared from conductivity water and Merck's highest-purity chemicals. The following solutions were used throughout the work:

For the cultivation of the nitrite-forming organism:

	grams
(a) Ammonium sulfate ($(NH_4)_2SO_4$)	1.0
Di-potassium phosphate (K_2HPO_4)	1.0
Sodium chloride (NaCl)	2.0
Magnesium sulfate ($MgSO_4$)	0.5
Ferric sulfate ($Fe_2(SO_4)_3$)	Trace
Water (conductivity)	1000.0
Magnesium carbonate ($MgCO_3$)	Excess

y

                                                                    grams

(b) Magnesium ammonium phosphate ($MgNH_4PO_4.6H_2O$)................ 2.0

    Di-potassium phosphate (K_2HPO_4)................................ 1.0

    Sodium chloride (NaCl).. 2.0

    Magnesium sulfate ($MgSO_4$)..................................... 0.5

    Ferric sulfate ($Fe_2(SO_4)_3$)...................................... Trace

    Water (conductivity)... 1000.0

    Magnesium carbonate ($MgCO_3$)................................. Excess

(c) Sodium ammonium phosphate ($NaNH_4HPO_4.4H_2O$).................. 3.4

    Potassium chloride (KCl)...................................... 2.0

    Magnesium sulfate ($MgSO_4$)..................................... 0.5

    Ferric sulfate ($Fe_2(SO_4)_3$)...................................... Trace

    Water (conductivity)... 1000.0

    Magnesium carbonate ($MgCO_3$)................................. Excess

For the cultivation of the nitrate-forming organism:

(d) Sodium nitrite ($NaNO_2$)... 1.0

    Sodium carbonate (Na_2CO_3)..................................... 1.0

    Di-potassium phosphate (K_2HPO_4)................................ 0.5

    Sodium chloride (NaCl).. 0.5

    Magnesium sulfate ($MgSO_4$)..................................... 0.3

    Ferric sulfate ($Fe_2(SO_4)_3$)...................................... Trace

    Water (conductivity)... 1000.0

Solution (a) was used for the cultivation of *Nitrosomonas* throughout most of the work and (b) and (c) used in comparison. Unless otherwise specified it will be understood that solution (a) was used.

All glassware was cleaned by treating it with strong alkali, strong sulfuric acid cleaning mixture, then washing in water, and finally rinsing in the conductivity water. The solutions were prepared in liter Erlenmeyer flasks, then pipetted into 150-cc. flasks and sterilized at 15 pounds pressure for 15 minutes. In solutions (a), (b) and (c), all the ingredients were added before sterilization except the magnesium carbonate, which was sterilized in a separate vessel and added to the solutions after they had cooled. During the first part of the work 25-cc. portions of the solutions were placed in the flasks; later on 15-cc. portions were used, because this afforded better aeration and therefore more rapid nitrification. In some cases the magnesium carbonate was added along with the other salts before sterilization and the ammonium sulfate sterilized in separate solution and added to the flasks by means of a sterile pipette.

Gypsum blocks. Gypsum blocks were prepared by mixing 2 gm. magnesium carbonate with 100 gm. of calcium sulphate, ($CaSO_4.H_2O$), and sufficient water to make a putty-like mass. This mixture was spread over a smooth glass surface to the desired thickness, marked out in squares, and allowed to dry. When dry the blocks were placed in petri dishes, sterilized, and the desired liquid medium added.

Washed agar. The agar was prepared by washing Difco agar in distilled water for several days and drying at 60°C. From the washed agar a 2.5 per cent solution was made, tubed in 10 cc. portions and sterilized in the autoclave

at 15 pounds pressure. The following solutions were prepared and sterilized in small portions, about 100 cc. per flask.

<div style="text-align:right">gm. per 100 cc.</div>

(1) Di-potassium phosphate (K₂HPO₄)................................. 1.5

(2) Sodium nitrite (NaNO₂)... 1.5
　　Sodium carbonate (Na₂CO₃)...................................... 1.5

(3) Magnesium sulfate (MgSO₄)...................................... 0.45
　　Sodium chloride (NaCl).. 0.75
　　Ferric sulfate (Fe₂(SO₄)₃)...................................... 0.02

(4) Ammonium sulfate ((NH₄)₂SO₄).................................. 1.5
　　Magnesium sulfate (MgSO₄)...................................... 0.75
　　Ferric sulfate (Fe₂(SO₄)₃)...................................... 0.02

(5) Sodium chloride (NaCl)... 3.0
　　Sodium carbonate (Na₂CO₃)...................................... 1.5

The agar was melted and cooled at 40°C. *Nitrobacter* plates were made by placing 1 cc. each of solutions (1), (2) and (3), in sterile petri-dishes, adding the desired inoculum, and then the melted agar. For the cultivation of *Nitrosomonas* 1 cc. each of solutions (1), (4) and (5) plus the agar were used. These stock solutions are about 15 times the strength of the usual medium; so that when 1 cc. of each of three are mixed with the 10 cc. of agar, all the nutrient salts are present in the proper proportions. The concentration is determined by the amount of agar added which may be controlled at will. The solutions prepared in this manner are convenient for use in agar or silicic acid gel. The nutrient salts may be added to the agar before autoclaving, but in this case the ammonium sulfate must be sterilized separately, otherwise a part of the ammonia will be lost in sterilization. The sodium carbonate should be handled in similar manner to prevent hydrolysis of the agar. Since these two ingredients must be handled separately it is well to prepare the solutions as stated above. In order to prevent the precipitation of the salts, it is necessary to handle the solutions as outlined. When preparing the plates it is desirable to prevent the 1-cc. portions of the different solutions from mixing before the agar is added to the plate; if this is attained the plate will contain no precipitated material and the slightest growth can be detected. If it is desirable to use magnesium carbonate as the base for the cultivation of *Nitrosomonas*, the sodium carbonate may be omitted from solution (5) and magnesium carbonate added to the plate at the time of pouring. Both magnesium and sodium carbonates were used in this work, but the latter gave better results because the plate remained clear and the colonies could be detected more readily.

No egg albumen was added to the agar to aid in clarification.

The agar was heated in the steamer for several hours, and filtered through macerated filter paper under pressure until it was clear.

Silicic acid gel. A convenient method for the preparation of silicic acid gel for use as a culture medium has never been devised. During the first part of the work the method of Stephens and Temple (54) was employed. The preparation of the medium by this method was very pleasing because of its ease, but the results secured from its use were very unsatisfactory. In no case did the nitrifying organism produce growth on this medium.

Doryland's (16) method was also tested but poor results were obtained. This was perhaps due to the high concentration of salts which resulted in an osmotic pressure that could not be withstood by the organisms. The Doryland method is very promising and should bring good results when worked out more in detail.

The method of Winogradsky also was employed, with collodion sacks as a dialyzing membrane, but gave results too irregular to be practical. Silicic acid secured by the Winogradsky method is difficult to handle because of its concentration; it often forms the gel in the dialyzing process, or gels when sterilized. It is interesting to note that the method of Stahel (53) is the same as that of Winogradsky except that the former used a sodium silicate solution with a specific gravity of 1.10, a 10 per cent solution, while the latter used a solution of 1.05 specific gravity, a 5 per cent solution. They each used a parchment membrane but the permeability must have been different because the resultant acid secured in each case was the same, or practically the same. The results bring out the fact that the membrane is the most important single factor, which is emphasized by Winogradsky.

The method of Beijerinck (7) was used with fairly satisfactory results. It is as follows: Merck's 40 per cent sodium silicate solution is diluted to 8 per cent, and a solution of hydrochloric acid prepared of such a strength that 1 cc. is just neutralized by 1 cc. of the 8 per cent sodium silicate solution, with methyl orange as indicator. A 5-cc. portion of the hydrochloric acid is then drawn from a burette into a sterile petri dish, and the amount of sodium silicate required to neutralize the acid added from a second burette. If the two solutions have been allowed to stand in the burettes for some time, they will be practically sterile. This method gives a beautiful gel in about 2 or 3 minutes. The silicic acid gel is then dialyzed by pouring sterile water over the surface until all the chloride has been removed. The entire procedure requires but a few hours for a large number of plates. The sterile concentrated nutrient solution is now poured on the surface of the plate and allowed to diffuse through the gel for some time; the excess is poured off and the surface of the gel flamed to insure that it is sterile. The plates are inoculated by placing a drop of the inoculum on the surface of the medium and spreading it around with the blunt end of a glass rod. The greatest objection to this method is the fact that the plates must be inoculated by streaking the surface, which is very difficult to do without disturbing the smooth surface of the gel, and the character of growth secured is not satisfactory for the study of single colonies.

It was desirable to have a gel which could be inoculated before solidifying, therefore dilute solutions were resorted to. The method used was primarily that of Schramm (52). Ninety cubic centimeters of Merck's 40 per cent sodium silicate solution are diluted to 1 liter with distilled water, and 125 cc. of concentrated HCl are diluted to 1 liter, and the two solutions poured together. It is necessary to pour the sodium silicate solution slowly into the hydrochloric acid solution, with vigorous stirring, or the silicic acid will precipitate immediately. The mixture, which should have no turbidity or cloudiness, is then placed in a dialyzer fitted with a parchment paper in contact with running water. After several days, 8 to 10, the mixture should be entirely free from chlorides, as determined by the silver nitrate test. It is then dialyzed further with several changes of distilled water, and a 10-cc. portion removed, evaporated to dryness, and ignited, and weighed to determine the percentage of silicon dioxide (SiO_2). If it is desirable to determine the purity of the acid, it may be done by moistening the residue with sulfuric acid, volatilizing the silica with hydrofluoric acid (HF), and reweighing. The dialyzed mixture usually gives an acid containing 1 to 1.5 per cent of silicon dioxide (SiO_2), Variations depending on the quality of the membrane used will occur. Since this solution of colloidal silicic acid is too dilute to produce the gel it must be concentrated, but if concentrated it will solidify on sterilization. This difficulty is met by sterilizing before concentrating. The acid solution is placed in a suction filter flask which is fitted with a thermometer and a capillary tube, which in turn is connected with a carbon filter plugged with cotton and is controlled with a pinchcock. The flask so arranged and containing the solution is then sterilized by the intermittent method. It is then attached to a suction pump and the pressure reduced to such an extent that the solution boils at 40 to 50°C. The capillary tube is opened slightly to permit a very slow stream of air to pass through the solution, but not sufficiently rapid to raise the pressure within the flask. The nutrient salts to be used with the silicic acid will determine how concentrated it must be to get the proper gel in the desired length of time. In this case the solutions given on page 26 were used, as previously described in connection with agar, and required an acid of 1.5 to 2.5 per cent of silicon dioxide (SiO_2). The addition of the salts causes the formation of the gel. Tests may be made as the silicic acid is concentrating by removing 10-cc. portions from the flask and adding the desired salts. When sufficiently concentrated to produce a gel of the desired consistency in a few minutes' time, the process is discontinued. In some cases it will be possible to sterilize this silicic acid solution after concentrating, either in the autoclave or by the intermittent method, without producing the gel. If this can be done the entire lot should be sterilized in suitable containers. If too concentrated to withstand sterilizing further, it must be used direct from the concentrating chamber. A clear solution is absolutely essential. If it takes on an opalescence or cloudiness it is always unsatisfactory because it will either not stand sterilization or will not form the gel when the desired salts are added.

It is a well known fact that the addition of electrolytes to a solution in the colloidal state tends to precipitate or bring about the gel condition. Since the nutrient solutions used in this work were all made up of electrolytes, no difficulty was experienced in obtaining the gel. If the medium is to contain organic nitrogenous matter, a higher concentration of the silicic acid must be used because of the protective action of the protein material. Stronger concentrations must also be used with a medium containing sugar because of the fact that carbohydrates in general produce an effect opposite in action to that of the electrolytes. "Reciprocal coagulation" also offers a means of obtaining a gel using more dilute silicic acid, but at present no work has been done in this direction.

It is important to note one interesting point in the preparation of silicic acid. Apparently under the same conditions the same silicic acid is not produced. On several occasions two portions of acid have been secured which contained the same percentage of silicon dioxide (SiO_2); one of these acids gave a gel when concentrated to 2 per cent of silicon dioxide (SiO_2) and would not stand sterilizing at that concentration, while the other was concentrated to 2.6 per cent and withstood heating in the autoclave several times at that concentration, and remained in the sol condition for several weeks. It gave a very beautiful gel of the desired consistency when mixed with the nutrient solutions. The results obtained bear out the fact that acids in various states of hydration may be secured, which act differently towards conditions commonly employed in media making. This would indicate a wider application of the method of preparation outlined by Doryland than has at present been worked out.

Each person using this medium must work out the details of the method of preparation on the general principles which have been laid down. The method will depend upon three factors—the silicic acid present, the concentration and the nutrient medium used. During the course of this work silicic acid gel plates containing the following constituents have been prepared along with the other ingredients.

> Heyden Nährstoff
> Mannite
> Asparagin and dextrose
> Dextrose and nitrate

Soil counts were made on these media with good results. *Azotobacter* developed readily on the mannite gel and produced the usual pigment. Large tubes of soft silicic acid gel also were prepared for the cultivation of plants and studying nodule formation, but the gel was not satisfactory for root development. Apparently the plant roots cannot penetrate this silicic acid gel medium.

In the following discussion the results of the work with *Nitrobacter* are presented first. The experience gained in the isolation of *Nitrobacter* aided materially in the isolation of the nitrite-forming organism.

The liquid cultures secured by inoculation with soil and carried in the enrichment process are referred to as "crude cultures," corresponding to the term "Rohkulturen" used by Winogradsky and other investigators.

Crude cultures of nitrobacter

Enrichment process. Flasks containing 25 cc. of the nutrient solution (d, p. 437) were each inoculated respectively with 0.5 gm. of garden, neutral and acid soil from widely separated sources. These flasks were incubated at 28°C., and after four or five days were tested with Trommsdorf's reagent until the absence of nitrites was noted. These cultures were then tested with di-phenylamine and sulfuric acid for the presence of nitrates. Wherever the nitrite was converted to nitrate, two or three loops were transferred to fresh flasks of the same medium. The mother culture was then covered with lead-zinc foil to prevent evaporation and held at room temperature, while the subcultures were incubated at 28°C. This procedure was continued throughout the work. The results are shown in table 1.

From the data in this table it is apparent that the time required for the complete oxidation of the nitrite varies with the different generations. Since the solutions were prepared in the same manner each time, and many of the generations are from one batch of medium, and the conditions of incubation are the same, it is safe to conclude that the variation in time for oxidation is due largely to the variation in the number of organisms carried by the loop inoculation. This difference in number will be further brought out in a later table. The cultures do not show any inclination to lose their virulence as a result of the enrichment process; they are still on hand and show their usual activity. The source of the culture has little influence on the time of oxidation, at least the three soils chosen for this test required practically the same time for oxidation.

The data in the table indicate that there are several cultures which required an exceptionally long period of time for oxidation. The long period of time required for oxidation of the neutral soil cultures F_{12} and F_{21} cannot be explained. Apparently the trouble in the F_{21} culture was not in that particular culture but in the F_{20} culture, because several F_{21} cultures were made before one was secured which would produce oxidation and this only after the lapse of 52 days.

Number of bacteria in enrichment cultures. Dilution counts were made to determine the number of organisms in soil capable of oxidizing nitrite in solution, and likewise the number present in the enrichment cultures. The

TABLE 1

Time required for oxidation of the sodium nitrite by the successive generations of nitrobacter

NUMBER OF GENERATION	TIME FOR OXIDATION		
	Neutral soil	Garden soil	Acid soil
	days	days	days
F	22	23	15
F_1	18	20	Date not recorded
F_2	9	6	9
F_3	10	9	10
F_4	8	12	7
F_5	9	11	12
F_6	10	9	12
F_7	11	8	10
F_8	11	8	7
F_9	14	9	7
F_{10}	16	10	10
F_{11}	13	10	8
F_{12}	35	10	14
F_{13}	12	10	19
F_{14}	10	11	30
F_{15}	17	15	15
F_{16}	14	12	15
F_{17}	16	30	14
F_{18}	23	12	17
F_{19}	19	20	34
F_{20}	21	10	15
F_{21}	52	15	13
F_{22}	14	9	21
F_{23}	12	12	22
F_{24}	11	8	21
F_{25}	10	27	8
F_{26}	8	16	5
F_{27}	13	9	27
F_{28}	9	11	7
F_{29}	11	7	7
F_{30}	10	6	8
F_{31}	10	8	7
F_{32}	10	6	7
F_{33}	9	6	7
F_{34}	7	7	10
F_{35}	7	7	7

results secured from the soil dilutions were unusually low. Flasks of the usual nutrient solution failed to produce oxidation when inoculated with 1 cc. of a 1:100 dilution from either the acid or neutral soil. The 1:10 dilution in each case produced oxidation after an incubation of 21 days. The results with the dilutions from enrichment cultures are shown in table 2.

The dilutions were made in sterile distilled water and 1 cc. of each dilution was used as an inoculum. From these dilution tests three facts are estab-

lished; first, soil contains relatively few active organisms capable of bringing about the oxidation of sodium nitrite in solution; second, the enrichment process increases the number of nitrite-oxidizing organisms to more than 1,000,000 per cubic centimeter; third, the time for oxidation depends upon .the number of organisms introduced in the inoculum. The rapidity of oxidation in the dilutions from F_{10} would indicate that 1,000,000 bacteria per cubic

TABLE 2

Number of nitrobacter organisms in the enrichment cultures

SOURCE	DILUTION 1 : x	DAYS FOR OXIDATION
	cc.	
	1 cc. undiluted	2
	10	3
	100	6
F_4 Acid soil	1,000	10
	10,000	12
	100,000	14
	1,000,000	No oxidation
	10,000,000	No oxidation
	100	8
	1,000	8
	10,000	14
	100,000	13
F_9 Neutral soil	200,000	13
	400,000	13
	600,000	15
	800,000	15
	1,000,000	18
	100	8
	1,000	13
	10,000	15
	100,000	18
F_{10} Neutral soil	200,000	19
	400,000	19
	600,000	20
	800,000	20
	1,000,000	20

centimeter was a very low figure in comparison to the number actually present. However, higher dilutions than this were not made.

Heyden agar plates were inoculated from these same dilutions in order to determine the number of foreign organisms in the enrichment cultures. Colonies developed on the plates from the highest dilutions. The colonies from the higher dilutions were apparently of only two or three types, one a small, round or oval colony, yellow in color, the other a milky colony, or light gray, having a dull appearance, and many times as large as the small yellow colony.

The small type of colony was much more numerous on all the plates. On the plates from the higher dilutions of the F_{10} enrichment only a few colonies developed, and these were apparently of the two types named. The plates from the lower dilutions produced abundant growth and showed the presence of putrefactive organisms.

As a result of these dilution experiments it is safe to conclude that not only does the enrichment process increase the number of nitrifying organisms but that it eliminates a large number of the other types. However, the enrichment process coupled with dilution will not give a pure culture of this organism.

The effect of silicic acid sol on enrichment cultures

A series of flasks of the usual sodium nitrite liquid medium were inoculated from an active enrichment culture. Pure colloidal silicic acid, 1.5 per cent of silicon dioxide, was then added in amounts of 2, 4, 6, 8, and 10 cc. per flask containing 15 cc. of liquid medium giving 0.176 per cent, 0.310 per cent, 0.428 per cent, 0.522 per cent and 0.60 per cent of silicon dioxide, respectively. These were then incubated at 28°C. Oxidation took place promptly in each, showing the silicic acid did not affect the activity of the organisms. This test was made because of the fact that a great many of the silicic acid plates inoculated gave slow growth and irregular results.

Effect of soil extract on enrichment cultures. Soil extracts from each of the three soils, neutral silt loam, acid silt loam, and garden soil high in organic matter, were prepared by heating 1 kgm. of soil with 1 liter of water in the steamer, then filtering and diluting the filtrate to 1 liter. These extracts were used for the preparation of the sodium nitrite nutrient solution for the cultivation of *Nitrobacter* and the flasks inoculated with an active enrichment culture. Oxidation resulted in each of the flasks with the usual promptness, showing the extracts not to be toxic. This is in accord with the results of Allen and Bonazzi (2) who also found that soil extract was not injurious to the enrichment cultures of *Nitrobacter*.

Isolation of Nitrobacter

Agar plates. Plates of washed agar were prepared as previously described, inoculated from one of the enrichment cultures, then placed in a moist chamber and incubated at 28°C. A period of 10 days to 2 weeks was necessary for the development of *Nitrobacter* on the plates. At the end of this time tests with Trommsdorf's reagent and diphenylamine, showed that the nitrite had been oxidized to nitrate. The growth was scarcely visible to the naked eye but when examined under the microscope numerous small light brown colonies could be seen. Some were round, others oval, and others three-cornered, depending upon their position in the agar. All the colonies were very small and almost any colony on the plate would answer the description of the

Nitrobacter colony given by one or more investigators. The deep colonies were regular in outline but the surface colonies were more or less irregular. A fresh portion of sodium nitrite was added to the plates by removing small portions of the agar and adding the sterile solution to the wells formed in the agar. The addition of nitrite caused the colonies to increase in size but no one type of colony developed faster than the other. At first it was assumed that all the colonies were *Nitrobacter*. This assumption, however, was not true as shown by transfers into liquid media.

Method of securing a single colony. The colonies were too small to make it practical to attempt the isolation of a single colony by means of the platinum needle. This difficulty was met by using a modification of the Barber (57) method of isolation. A small moist chamber, open at one end and the top, was made by sealing glass slides together with balsam. A capillary tube was then prepared as described by Barber, except that in this case it was not necessary to secure such a fine point. A long sterile cover glass was then slipped under the agar on the plate and a strip removed. This was allowed to remain on the glass and was trimmed to proper size, then the cover glass inverted over the moist chamber in such a manner that the agar was suspended on the slide, as in the hanging drop. The capillary tube was fitted into the mechanical holder and the point brought into focus under the lens, the 16-mm. objective being used. The capillary pipette was lowered and the moist-chamber brought into place under the objective. The chamber was moved about until a desirable colony was located and brought into the center of the field, then the pipette brought upward by the mechanical holder until the point came in contact with the colony. The colony readily moved into the pipette by capillarity—often the opening in the pipette was as large as the colony itself. The colony thus secured was transferred to the usual nutrient solution and growth measured by oxidation.

This process seems simple and yet many attempts failed before the organism was secured in pure culture. Usually the organism secured from the agar in this manner failed to oxidize the nitrite in the solution, or if oxidation took place subsequent inoculation into bouillon resulted in growth. Many colonies in isolated positions on the medium were secured in this manner and when inoculated into the usual nutrient solution produced oxidation, yet the bouillon test after oxidation showed contamination. Either the contaminating organism was very closely associated with the nitrate-forming organism and the colony characteristics were so nearly alike they could not be differentiated, or the nitrate-forming organism produced growth in bouillon as claimed by Beijerinck (7). A large percentage of the colonies transferred to the nutrient solution resulted in no oxidation of the nitrite, yet a very small piece of the agar 5 mm. square readily produced oxidation when placed in the same solution. This would indicate that a large inoculum was necessary as is the case with certain other organisms. Two colonies which appeared identical were chosen, one transferred to a nutrient solution, the other to bouillon. Neither

gave evidence of activity, the nitrite remaining unchanged in the solution, the bouillon remaining clear. This would indicate a third organism, which would neither grow in bouillon or oxidize the nitrite in the nutrient solution, as claimed by Stutzer and Hartleb. However, this is not considered to be true in this case, but is explained by the fact that growth in bouillon results only from use of a large inoculum. The small percentage giving oxidation of the nitrite may be explained in a similar manner. This will be further discussed with the results of the *Nitrosomonas* study.

To narrate all the difficulties which arise in the isolation of *Nitrobacter* would be a difficult task and without interest to the reader. The following translation from the report of Stutzer and Hartleb (57) quite accurately expresses the sentiment of the author in regard to the difficulties of isolation, more so in connection with the isolation of *Nitrosomonas* than with *Nitrobacter*. However, this quotation deals with the isolation of *Nitrobacter*.

Simple as the described method for the isolation of the nitrate former may appear, which varies little in its technique from the customary bacteriological methods of securing a pure culture, and differs from these mainly only in the application of other nutrient media, yet the practical application of it is very round-about and requires much time, because very often difficulties arise which make the undertaken work absolutely useless, or if one believes that he is at his goal a new difficulty suddenly appears. For instance, such a difficulty was found in the separation of the two organisms which would not grow in bouillon, *Hyphomicrobium* and *Nitrimicrobium*. As long as we did not know that several bouillon sterile organisms occurred in the soil we were inclined to consider the two kinds mentioned as identical, inasmuch as also their colony formation and their action on nitrite agar show a surprising similarity. . . . . And we may comfort ourselves with the thought that up to this time no other investigator has been successful by arriving completely at his goal in this special field.

Silicic acid gel plates. The results secured from the use of silicic acid gel were more satisfactory in the earlier part of the work of isolation than those secured from the use of washed agar. However, the same disappointment may be felt when the isolation is begun. The silicic acid gel is not well adapted to the cultivation of organisms other than those requiring an inorganic medium, which makes it a very valuable solid medium for the cultivation of the nitrifying organisms. After an incubation of 10 days to 2 weeks the nitrite in the medium was all oxidized. When examined under the microscope numerous very small light brown colonies were seen, some of which were not *Nitrobacter*, but after the selection of a great number as described above, a pure culture was obtained. The colonies remained much smaller than those on the agar, although more nitrite was added repeatedly.

Tests for purity. The fact that colonies from agar or silicic acid gel, secured as above outlined, resulted in oxidation of the nitrite in solution, was not taken as an absolute proof of the purity of the culture. Further tests were made by inoculation into bouillon and upon agar slopes. The bouillon tubes were incubated 8 to 10 days because the growth of the contaminating forms devel-

oped very slowly. Agar containing 3 gm. of beef extract and 5 gm. of peptone per liter gave better results than bouillon. The growth on the agar was visible more quickly than in bouillon. The type of growth is very slight in comparison to the growth secured with common soil organisms. When bouillon was used as the test for purity, 0.5 cc. of the culture was used as inoculum. It was found that loop inoculations often fail to show the presence of the contaminating organisms. The contamination usually consisted of two organisms which also occurred with *Nitrosomonas* and will be described in connection with the discussion of that organism.

Pure culture characteristics of Nitrobacter

Liquid cultures. When the nutrient solution used in the *Nitrobacter* enrichment process was inoculated with a pure culture of the organism, complete oxidation of the nitrite resulted in about 7 days. At the end of that time, the solution contained no visible growth. After the addition of several portions of sterile 1.5 per cent sodium nitrite solution, with subsequent oxidation of each, a very slight flocculent material began to accumulate. This material always settled to the bottom of the solution, and in no case formed a film on the surface or developed on the sides of the flask. When removed and stained with carbol fuchsin it was found to consist of masses of the *Nitrobacter* cells together with disintegrated material. The amount of this material was small. The oxidation of the nitrite solution continued with the addition of each portion until the concentration of nitrate became surprisingly high.

The organisms were readily stained with carbol fuchsin. Loeffler's methylene blue and aqueous gentian-violet were tried without success. The organism is distinctly oval in shape, commonly found in single cells or in pairs, about 0.6 to 0.8 μ wide and 1 to 1.2 μ in length. It is non-motil and non-sporeforming. The thermal death-point is between 56° and 58°C. Stains of the typical forms are shown in plates 2 and 3.

Colonies on washed agar. Agar plates inoculated with pure cultures of the organism produced colonies which were first identified under the microscope after an incubation of 7 to 10 days at 28°C. They appeared as very small light brown colonies more or less rounded in outline. After the nitrite was completely oxidized to nitrate, usually 10 days to 2 weeks or even longer, the colonies developed somewhat larger. They then appeared darker in color, round or three-cornered in shape, and with a regular outline. The surface colony was occasionally regular in outline but was more often irregular and spreading. The deep colony at this stage was 30 to 50 μ in diameter; the surface colony somewhat larger, 50 to 150 μ in diameter, and with a tendency to spread. The deep colony appeared homogeneous and somewhat shiny, the surface colony slightly granular and dull. The typical deep colony on agar is shown in plate 1.

Colonies on silicic acid gel plates. The colonies on the silicic acid gel were very similar to those on washed agar, being somewhat more dense and not as large. They had the characteristic light brown color, later becoming dark brown. When silicic acid gel plates were prepared after the method of Beijerinck and inoculated by streaking the surface, growth was more profuse and resembled that on the agar slope. To the naked eye it appeared as very scanty gray streaks over the plate. When examined under the microscope the streak was found to be made up of a mass of growth, light brown in color, around the edge of which could be seen many very small light brown colonies. When small portions of the gel were removed and sterile sodium nitrite added to the wells thus formed, the growth increased and became a characteristic dark brown color when examined under the microscope. To the naked eye the growth remained a light gray color throughout the period of activity of the organisms.

Washed agar slopes. When a slope of washed agar containing the nutrient salts was inoculated with a pure culture in liquid medium, growth was visible after a period of 4 to 5 days, and after a period of 7 to 10 days formed a scanty greyish streak on the surface. Growth was more uniform after a few transfers, the first growth secured being somewhat scattered. When the slopes were inoculated from cultures in liquid media the resultant growth was often in the form of numerous very small colonies on the surface of the agar. Continuous growth was secured from this by streaking the surface of the slope with the platinum needle, thus joining the colonies. The usual nutrient solution when inoculated from the slope was promptly oxidized.

Accumulation of nitrate in liquid cultures. Two 1-liter Erlenmeyer flasks each containing 100 cc. of the usual sodium nitrite nutrient solution were inoculated with a pure culture of *Nitrobacter* and incubated at 28°C. When tests with Trommsdorf's reagent showed no nitrite to be present 1 cc. of sterile 10 per cent sodium nitrite was added to each flask. After the oxidation of the first portion of nitrite the solutions remained quite clear, but after the addition of one or two portions of the 10 per cent solution the flaky growth began to appear. Stains then showed the presence of an enormous number of the *Nitrobacter* cells. The flaky material developed for some time, then remained at about the same stage without further development. Each time the nitrite was oxidized more was added and the process continued until no further oxidation could be secured. Analysis of the solutions at the end of the period showed them to contain 502 and 527 mgm. of nitrogen, respectively, as nitrate per 100 cc. of solution. Stains at a late stage of oxidation showed the presence of fewer organisms than after the addition of the third portion of the nitrite solution. After all oxidation ceased transfers were made to fresh media and oxidation resulted promptly, showing that the organisms were not killed by the products of their own metabolism but a concentration was reached beyond which no further activity took place.

Thermal death-point. The thermal death-point was determined by use of actively oxidizing pure cultures in liquid media. Tubes containing 10 cc. of a 0.7 per cent sodium chloride solution were placed in the water baths at various temperatures and held until of uniform temperature throughout. Then 0.5 cc. of the culture was pipetted into each tube, the contents thoroughly shaken, and held at the temperature for 10 minutes, and then 1 cc. was drawn from the tube and used as inoculum in the usual sodium-nitrite-nutrient solution. All tests were run in duplicate at different times, different cultures being used. The thermal death-point was found to be between 56° and 58°C.

Longevity. Two samples of soil were available which had been on hand in the laboratory since October 28, 1910. This soil had been taken from the field on that date, air-dried, passed through a 100-mesh sieve, and then stored in tightly stoppered bottles. This soil was used as inoculum in the usual nutrient solution February 3, 1917. Oxidation of the nitrite resulted promptly in each flask inoculated, showing the resistance of the organisms to drying.

Ten grams of soil were placed in each of a number of test tubes and sterilized in the autoclave at 10 pounds pressure for 1 hour on each of three consecutive days. One cubic centimeter of sterile sodium nitrite was then added to each tube. The tubes were then divided into two series; one series was brought to optimum moisture content, approximately, and the other series was unchanged. The tubes of the series with the optimum moisture content were then sealed to prevent loss of moisture while those of the first series were left with cotton plugs. Both series of tubes were then held at room temperature to determine how long the organisms would retain their vitality, as determined by their activity when inoculated into the usual nutrient solution.

The organisms remained active in all the tubes, both sealed and plugged with cotton, for a period of 4 to 5 months. They then began to show less activity in that it required a longer period of time for the oxidation of the nitrite when inoculated into the nutrient solution. The last test was made 17 months after the tubes were stored and at that time the organisms were present and still able to oxidize. Bouillon tubes inoculated from the soil remained free from all growth.

Crude cultures, Nitrosomonas

Enrichment cultures. The flasks containing the ammonium sulfate nutrient solution (a, p. 436), were each inoculated with 0.5 gm. of the three soils previously described. They were then incubated at 28°C., and tested every second or third day with Trommsdorf's and Nessler's reagents. When the test with Trommsdorf's reagent gave a deep blue color loop transfers were made to fresh media, and the process continued. The results of the enrichment process are shown in table 3.

From the data in the table it is apparent that the time required for the oxidation of the ammonia in the different generations is variable. In general the

TABLE 3

Time required for the oxidation of ammonium sulfate by the successive generations of nitrosomonas

GENERATION NUMBER	TIME FOR OXIDATION		
	Neutral soil	Garden soil	Acid soil
	days	*days*	*days*
F	7	7	7
F₁	8	21	8
F₂	5	9	5
F₃	8	10	15
F₄	6	53	4
F₅	6	61	6
F₆	6	6	6
F₇	6	8	6
F₈	6	7	6
F₉	7	7	6
F₁₀	8	16	7
F₁₁	9	12	8
F₁₂	8	20	9
F₁₃	8	15	8
F₁₄	6	10	8
F₁₅	13	9	6
F₁₆	10	11	13
F₁₇	20	40	10
F₁₈	9	6	11
F₁₉	6	7	10
F₂₀	9	7	6
F₂₁	10	4*	6
F₂₂	9	6	9
F₂₃	5	6	9
F₂₄	16	7	9
F₂₅	16	6	5
F₂₆	10	4	19
F₂₇	8	7	31†
F₂₈	7	7	9
F₂₉	9	7	7
F₃₀	7	7	8
F₃₁	7	7	8
F₃₂	7	8	6
F₃₃	6	8	7
F₃₄	4	8	7
F₃₅	7	7	7
F₃₆	8	7	7
F₃₇	7	10	7
F₃₈	7	7	6
F₃₉	7	8	4
F₄₀	8		7
F₄ₐ	8		7

* Garden soil series.   15 cc. medium substituted for 25 cc. and maintained in rest of cultures.

† First F₂₇ failed to oxidize the ammonia, second required 31 days.

TABLE 3—*Continued*

GENERATION NUMBER	TIME FOR OXIDATION		
	Neutral soil	Garden soil	Acid soil
	days	*days*	*days*
F_{42}	7		7
F_{43}	7		7
F_{44}	7		7
F_{45}	10		8
F_{46}	6		8
F_{47}			6
F_{48}			7
F_{49}			6
F_{50}			10
F_{51}			6
F_{52}			7

time becomes more uniform in the higher generations, as was found with the *Nitrobacter* cultures. The fluctuation in most cases is probably due to the variation in the number of nitrite-forming organisms transferred. No explanation can be given for the extreme length of time required for the oxidation of the ammonium salt in a few of the cultures, since all media were prepared in the same manner and all cultures were handled alike. The garden soil series was very variable until the F_{21} generation. At this point 15 cc. of the nutrient solution was substituted for 25 cc. previously used. The increased surface, in proportion to the depth of the solution, was responsible for the more vigorous oxidation of the ammonium salt. In the acid soil series the F_{27} generation failed to oxidize the ammonium and it was only after several transfers were made that a culture was secured which was active. The point of interest in this connection is the fact that there was no loss of oxidizing power from transfer to transfer as the cultures were continued. This is not in accord with the results secured by Allen and Bonazzi (2), who found that the cultures became less active in the successive transfers. In their first paper the cultures became so weakened after three or four transfers that they were forced to discontinue the process.

After a few generations of enrichment cultures had been secured, stains with carbol fuchsin were made to determine the relative number of organisms present. In the majority of cases the stained preparations showed relatively few organisms but if the material for staining was taken from the bottom of the flask numerous clumps of organisms could be seen. These were in general about 0.9 to 1 μ wide and 1.2 to 1.5 μ long, rounded or oval, and uniformly stained. Few other types of organisms were present, but if the smears were examined carefully a very small rod form could be found and also an occasional clump of a very small coccus form. Sterile ammonium sulfate solution was then added to the cultures and was promptly oxidized to nitrite in two or three days, and each successive portion added was likewise oxidized. It

was necessary to add more magnesium carbonate from time to time as the concentration of nitrite increased. No growth in the culture solutions was visible until the nitrous acid formed had rendered the magnesium carbonate soluble, and then only a very slight flaky material could be seen. In no case did the organism grow on the surface of the solution or on the sides of the flask. When stains were prepared from the cultures after the oxidation of several added portions of ammonium sulfate, the number of organisms was found to have increased enormously. The stains showed the same type of organism previously observed but in much greater numbers, both in clumps and scattered as free cells over the field. The contaminating organisms were either in such a minority or so nearly resembled the nitrite-forming organism that the stain indicated the cultute was pure. However, this was not the case, as will be explained later. The nitrite content gradually increased, as each successive portion of ammonium sulfate was oxidized, accompanied by a large increase in the number of organisms up to about the seventh or eighth addition, beyond which the cells began to disintegrate, probably because of the high concentration of nitrite. After the addition of eight or nine portions of ammonium sulfate solution the time required for oxidation increased materially and after the addition of 12 to 15 portions the process was entirely inhibited.

Flasks containing the nutrient solutions of magnesium ammonium phosphate (b, p. 437) and sodium ammonium hydrogen phosphate (c, p. 437) were inoculated from one of the enrichment cultures, and after oxidation of the ammonia had occurred transfers were made in the usual manner. These cultures were carried through a number of generations in order to determine whether or not there was any advantage in the use of these salts as the source of ammonia. The results showed that the time required for the oxidatio n of these salts was much the same as for ammonium sulfate, and if there was any advantage it was in favor of the ammonium sulfate solution.

Number of bacteria in enrichment cultures. In order to determine whether or not the enrichment process was actually increasing the number of nitrite-forming organisms dilution counts were made from the cultures. These dilutions were made immediately after the oxidation of the ammonium sulfate, and before the addition of a second portion. The culture was shaken vigorously in order to get the contents well mixed, and 1 cc. transferred by means of a sterile pipette to a 99-cc. water blank. Successive transfers were made in a similar manner, thus securing the dilutions indicated in table 5. In the case of the F_{31}, acid soil, the dilutions were made direct in the nitrifying media, transferring 1 cc. from each flask to the next. One cubic centimeter of each dilution in the water blanks was used as inoculum in the usual nutrient solution, and in this manner the presence or absence of *Nitrosomonas* in that dilution determined. At the same time Heyden agar plates were inoculated from the dilutions and incubated at 28°C. The results of the *Nitrosomonas* counts are shown in table 4.

From the data in the table it is apparent that the number of nitrite-form—
ing organisms in the cultures has increased from enrichment F_4 to enrichmen███
F_{33}. It is also apparent that the time required for the oxidation of the ammoni═══
was somewhat dependent on the number of organisms used as inoculum. The═══
highest count obtained was 19,000,000, which is about the total number of═══
organisms usually found in 1 gm. of soil as determined by the plate method ══

Colonies developed on the Heyden Nährstoff agar plates from all the dilu══
tions, showing contaminating organisms to be present in numbers equall══

TABLE 4

Number of nitrosomonas organisms in the enrichment cultures

GENERATION NUMBER	DILUTION 1 : x	DAYS FOR OXIDATI══
F_4. Acid soil; dilutions in distilled water; 12/28/15 . . .	none, 1 cc.	2
	10	3
	100	6
	1,000	10
	10,000	12
	100,000	14
	1,000,000	No oxidatio═══
	10,000,000	No oxidatio═══
F_{11}. Acid soil; dilutions in saline; 2/10/16	100	7
	1,000	8
	10,000	14
	100,000	13
	200,000	13
	400,000	13
	600,000	15
	800,000	15
	1,000,000	18
F_{10}. Neutral soil; dilutions in saline; 2/10/16	100	8
	1,000	8
	10,000	9
	100,000	lost
	200,000	15
	400,000	18
	600,000	15
	800,000	19
	1,000,000	22
F_{33}. Acid soil; dilutions in nitrifying solution; 11/7/16 .	100	6
	2,500	10
	62,500	11
	125,000	13
	781,000	16
	1,562,600	16
	19,031,050	18
	38,062,500	No oxidation

TABLE 4—*Continued*

GENERATION NUMBER	DILUTION 1:x	DAYS FOR OXIDATION
	100	5
	1,000	7
	10,000	8
	100,000	10
	200,000	10
	500,000	11
F₂. Acid soil; dilutions in saline; 11/17/16..........	800,000	12
	1,000,000	14
	2,000,000	15
	5,000,000	17
	8,000,000	17
	10,000,000	19
	20,000,000	No oxidation
	50,000,000	No oxidation

as great as or greater than *Nitrosomonas*. The plates from the F_4 generation showed a higher number of colonies than those from F_{10}, indicating an elimination of undesirable types due to the enrichment process. The plates from the lower dilutions showed five or six types of colonies, those from the higher dilutions developed 4 to 20 colonies each of apparently only two types, one a very small, round or oval colony, yellowish brown in color, the other a light greyish colony of dull appearance, very round in shape, and many times as large as the small yellow colony. The small colony was far more numerous than the larger type, and sometimes developed a bright yellow color when seen under the microscope. From the results of these plates it seems that only two organisms are present in the enrichment cultures in as great numbers as *Nitrosomonas*. These two organisms will be discussed later.

The results of these dilution counts are much the same as those secured with the *Nitrobacter* cultures and the same conclusions may be drawn from their study. Not only does the enrichment process increase the number of desirable organisms but it eliminates a high percentage of the undesirable types.

Effect of sodium chloride on enrichment cultures. The poor results secured by the use of silicic acid gel prepared after the method of Stephens and Temple (54) were attributed to too high a concentration of sodium chloride. Flasks containing the usual ammonium sulfate nutrient solution and sodium chloride at various concentrations were each inoculated from an active enrichment culture, in order to determine the amount of sodium chloride which would exert toxic action on nitrite formation. The results are shown in table 5.

The solution used for the cultivation of *Nitrosomonas* throughout the work contained 0.2 per cent sodium chloride. From the data in the table it is seen that concentrations above 0.8 per cent greatly inhibited nitrite formation, and concentrations above 1 per cent prohibited oxidation. The data indicate that silicic acid gel prepared after the method of either Stephens and Temple

TABLE 5

Effect of sodium chloride on nitrosomonas enrichment cultures

GENERATION	SODIUM CHLORIDE PERCENTAGE	DAYS FOR OXIDATION
F₈. Acid soil; 2/8/16...........................	0.2	5
	0.2	5
	0.5	9
	0.5	10
	1.0 ·	No oxidation
	1.0	No oxidation
F₉. Neutral soil; 2/8/16.....................	0.2	5
	0.2	5
	0.5	12 to 15
	0.5	12 to 15
	1.0	No oxidation
	1.0	No oxidation
F₁₆. 4/14/17.................................	None	17 ⎫ very
	None	17 ⎭ weak
	0.05	9 ⎫ fairly
	0.05	9 ⎭ strong
	0.10	9 strong
	0.10	9 strong
	0.20	9 strong
	0.20	9 strong
	0.40	9 strong
	0.40	9 strong
	0.60	9 strong
	0.60	9 strong
	0.80	12 strong
	0.80	15 strong
	1.00	20 ⎫ very
	1.00	20 ⎭ weak
	1.50	No oxidation
	1.50	No oxidation
	2.00	No oxidation
	2.00	No oxidation

or Doryland is not adaptable to the cultivation of *Nitrosomonas*. The F$_{16}$ culture withstood a higher concentration of the sodium chloride than the F$_9$ culture, which indicates that continual cultivation in the liquid medium has increased the resistance of the organism to sodium chloride.

Effect of silicic acid on enrichment cultures. Silicic acid, containing 1.5 per cent of silicon dioxide (SiO$_2$) was added to the flasks containing the nutrient medium, in quantities of 2, 4, 6, 8 and 10 cc., respectively. Thus the flask containing 2 cc. of the silicic acid sol had a total volume of 17 cc. of solution while the flask receiving 10 cc. of the silicic acid had a volume of 25 cc. of solution, giving 0.176 per cent, 0.316 per cent, 0.428 per cent, 0.522 per cent and 0.600 per cent of silicon dioxide, respectively. The flasks were then inoculated from an active enrichment culture. Oxidation resulted promptly in each flask. Varying quantities of pure silicic acid in the gel state were then added to each of another series of flasks which were then inoculated. Oxidation resulted in each flask in the usual time. These results showed that the

TABLE 6

Effect of soil extract on enrichment culture of nitrosomonas

EXTRACT	K$_2$HPO$_4$ + or −	OXIDATION
Neutral soil...................................... {	+	0
	−	0
Acid soil.. {	+	+
	−	−
Garden soil...................................... {	+	++
	−	++

   0 No nitrite formation.
   + Fair nitrite formation.
   ++ Very strong nitrite formation.

silicic acid either as sol or gel had no toxic action on nitrite formation in solution. Bonazzi found the same thing true with his cultures.

Effect of soil extract on enrichment cultures. Soil extracts from each of the three soils, neutral silt loam, acid silt loam, and garden soil heavy in organic matter, were prepared by heating 1 kgm. of soil with 1 liter of water in the steamer for 1 hour. These were then filtered and washed until the filtrate from each was 1 liter in volume. These extracts were then each divided into two lots, one of which received 1 gm. di-potassium phosphate (K$_2$HPO$_4$) per liter, while the other received no treatment. These extracts were then used as the nutrient medium, after the addition of ammonium sulfate and magnesium carbonate in the proper proportion. Both these ingredients were added after the sterilization of the extract. The flasks were then inoculated from an active enrichment culture and tested regularly for the first oxidation of the ammonia. The results are shown in table 6.

The cultures prepared with the garden soil extract gave oxidation as readily as or more readily than those in the usual ammonium sulfate nutrient solution. This was unexpected since the extract was deep brown in color from the soluble organic matter. The acid soil extract was much lighter in color and the neutral soil extract was nearly colorless. The absence of soluble plant-food rather than the toxic effect of the organic matter explains the results of no oxidation in the first two extracts. The garden soil had an abundance of soluble plant-food as well as the large amount of organic matter.

Isolation of Nitrosomonas

Agar plates. The isolation of the organism by use of the plate method was accomplished only by the use of super-enrichment cultures; that is, cultures which had received several additions of ammonium sulfate with subsequent oxidation of each. A drop of sterile water was placed in each of several petri-dishes and a loop of the solution taken from the desired culture and mixed with the water in the first plate, then a loop from this to the second plate, etc., thus securing a greater dilution with each successive plate. The nutrient salts in sterile concentrated solution were then added as previously described, the agar poured into the plate and the contents thoroughly mixed, and then the plate incubated in incubating cylinders at 28°C. The plates were tested at intervals by removing a small piece of the agar and placing it in Trommsdorf's reagent. In case the nitrite formers were active, which in general required an incubation of 2 to 4 weeks, the test would give a deep blue color. When the plates were examined before there was any nitrite formation they were found to contain many colonies, scarcely visible to the naked eye, but visible under the 16-mm. objective. The colonies appeared light brown in color, rounded or oblong in shape, and usually with a smooth outline. When colonies were removed and stained they were found to contain two types of organisms, one a very minute bacillus, the other an oblong form resembling *Nitrosomonas* but considerably smaller. When inoculated into bouillon, growth resulted in two or three days. The colonies on the plate varied in size from 30 to 150 μ.

After the plate had shown nitrite formation but little change in growth could be detected. The same type of colonies predominated and a few more had developed, which also were very small and very light brown in color. When isolated colonies were removed from the plate by means of the Barber apparatus and placed in the usual liquid medium, oxidation resulted in about one case out of twelve or more. When stains were made from those showing oxidation, apparently only the *Nitrosomonas* form was present, but when examined very closely the small bacillus form could be found. Bouillon was used as a test for purity since Winogradsky states that neither of the nitrifying organisms would produce visible growth in that medium. When bouillon tubes were inoculated from the culture in solution, which was secured from

the agar colony, conflicting results were secured. The bouillon tubes were inoculated with one or two loops from the active culture. Some of the tubes thus inoculated produced growth while others did not. Those giving no growth were considered pure and transfers were made to fresh media. The subcultures showed prompt oxidation and when bouillon was inoculated from them some produced growth and others did not. These results were very confusing. If the mother culture proved "bouillon sterile" why should the daughter cultures show growth in bouillon? It was then found that if 0.5 cc. of each culture was used as inoculum for the bouillon, growth resulted in every case. From the results it appears that the cultures were not pure but contained another organism which was present in such small numbers in comparison to the number of *Nitrosomonas* cells that occasional loops could be secured which contained none of the contaminating form. Working on this supposition, a great number of dilution tests were made and media inoculated from each dilution. The results showed that the cultures contained a very high number of nitrite-forming organisms, in some cases between 10 and 20 million per cubic centimeter of solution. Each flask inoculated from the respective dilutions was promptly inoculated into bouillon as soon as it had shown oxidation, 0.5 cc. of the solution being used as the inoculum. Some gave growth in bouillon while others did not. The results indicated that the former supposition is not correct, but that the contaminating form was present in larger numbers than *Nitrosomonas* or was so closely associated with it that the ordinary dilution methods would not separate them. In certain cases the mother culture would not produce growth in bouillon while the F_1 cultures would produce growth in bouillon and similar results with the F_2 generation. This peculiarity has been noted several times.

Only one, or occasionally two, types of growth would appear in the bouillon tubes inoculated from the cultures. Later in the work only a very small bacillus form was found. If the cultures contained only two contaminating forms they should be separated easily from *Nitrosomonas* by the plate method, since the latter organism is present in such great numbers. Many agar plates were inoculated from these cultures in various dilutions. Some of the plates produced colonies and prompt oxidation of the ammonium salt while others gave entirely negative results. When studied under the microscope the plates were found to contain numerous small brown colonies, the same as those previously described, and transfers from these colonies to the nutrient solution gave the same results as those secured before; that is, about 10 per cent or less gave oxidation of the ammonium salt, and these gave sporadic growth when inoculated into bouillon.

When dilutions were made from the cultures in an effort to eliminate the contaminating form, bouillon tubes as well as the ammonium sulfate nutrient solution were inoculated from each of the dilutions. In the majority of the cases the bouillon tubes remained free from growth after 2 weeks' incubation, even from the lower dilutions, while the flasks of the nutrient solutions, inoc-

ulated from the same dilutions, gave prompt oxidation and in many cases subsequent growth in bouillon.

Silicic acid gel plates. The inexplainable results secured from the use of agar as the base for a solid medium led to the use of silicic acid gel as a substitute. The method of preparing this as a medium in this work has been previously described. The results obtained were somewhat more encouraging. This medium when inoculated with an active nitrite-forming culture showed the formation of nitrite after an incubation of 3 to 4 weeks. Growth was scarcely visible without the aid of a microscope, but when examined under the 16-mm. objective numerous very small light brown colonies could be seen. These colonies did not develop as large as those on the agar but remained very small. At first they were round and regular in outline but after the addition of more ammonium sulfate to the plate and longer incubation, scattered cells could be seen around the colonies. Close examination showed that the colony had become so dense that it had bursted apart and the single cells were moving outward from the center. The colonies were very difficult to remove from the medium and extremely difficult to tear apart. If a colony was removed by the method previously described, placed on a slide and stained with carbol fuchsin, in the majority of cases no organisms could be found. Even when small chunks of the silicic acid gel containing several of the colonies were smeared on the slide and stained, no organisms could be found or if so they were in such dense clumps that single organisms could not be studied. For a while this was very confusing but after a time an explanation was found. The organisms clung tightly to the silicic acid and in the fixing they became attached to the small particles of silicon dioxide (SiO_2). This conclusion was formulated from the discovery that the colonies could be stained directly on the gel. Carbol fuchsin was poured directly on the surface of the gel, allowed to remain from 40 to 80 seconds, then washed off with a slow stream of water. If it was not desirable to stain the whole plate a small portion of the gel was removed by means of a thin glass slide and stained in the same manner. The colonies were deeply stained while the gel contained no noticeable stain. Photomicrographs of the colonies stained by this method are shown in plates 4 and 5.

The colonies of *Nitrosomonas* were not always as described above. It was very difficult to secure silicic acid gel plates of the same physical composition, and the consistency of the gel determines the nature of the colony. On the softer gels the colonies were not so compact. The colonies appeared first as small refractive bodies with sharp outline and later they became light brown in color and continued to darken until fairly dark brown. As they became older the outline became more irregular and a number appeared as those shown in plate 5. To the naked eye the colonies appeared light gray in color.

Other colonies not *Nitrosomonas* were seen on the plates inoculated from the crude cultures, which were very similar in size, color, and shape to the *Nitro-*

somonas colonies. These non-nitrifying colonies were small, rounded or oblong in shape, and brown in color. A few of the colonies had a granular appearance, a sharp outline within which the organisms were somewhat loosely arranged approaching a more dense granular center. This type of colony was usually larger than the others and was found on the surface.

When colonies were selected from the gel and inoculated into the usual ammonium sulfate nutrient solution, nitrite formation resulted with great irregularity. A small percentage produced oxidation of the ammonia, probably a slightly higher percentage than was secured from the agar colonies. Subsequent tests with bouillon gave the same results that were secured from the use of agar. One interesting fact which had not been previously noted in the use of agar was brought out from the use of the silicic acid gel. On several different occasions plates were secured which contained a large number of colonies and which gave an active oxidation of the ammonium salt. These colonies answered in every detail the description of the *Nitrosomonas* colony given by Winogradsky and were identical with his photographs. The colonies were transferred to the usual ammonium sulfate medium but produced no oxidation, and when transferred to bouillon they produced no growth. Great numbers were then scooped from the plate and used as inoculum into each medium, with negative results. This fact was later noted in many cases when agar plates were used. When stained the organisms were not typical of *Nitrosomonas* as found in the active state in liquid medium but were smaller, yet they still maintained their characteristic shape.

There was on hand in the laboratory one culture, no. X, which produced active oxidation but would produce no growth when inoculated into bouillon repeatedly, yet its daughter cultures, after oxidation of the ammonia, produced growth in bouillon. One of the members of the department wished to test the results secured with this culture. His tests with bouillon likewise gave negative results. He then inoculated 10 parallel flasks of fresh ammonium sulfate medium from this culture, first running controls on the medium used, and when the ammonium salt had been oxidized tested for purity by the inoculation of bouillon. After oxidation of the ammonium sulfate more was added and the bouillon test repeated, etc., until no further oxidation could be secured. Each of the 10 cultures produced growth in bouillon throughout the entire length of the experiment. Fourteen additions of ammonium sulfate were made and likewise 14 bouillon tests, each of which produced growth. After no further oxidation could be secured 6 of the cultures were analyzed for nitrite content. They contained the following amounts of nitrogen as nitrite, calculated per 100 cc. of solution culture: 1 = 184 mgm., 2 = 170 mgm., 3 = 208 mgm., 4 = 206.6 mgm., 5 = 106.6 mgm., 6 = 180 mgm.

The results secured from this experiment show that the bouillon test was not influenced by the nitrite content of the solution used as inoculum. In all cases 0.5 cc. of the culture was used as inoculum for 10 cc. of the bouillon.

This sporadic growth in bouillon may be described best by the results of one series which are shown in figure 1. Several of such series of tests have been made and this particular one affords a representative example of the results secured in each case. This chain began with culture X which was just described. This culture oxidized each added portion of ammonium sulfate until the solution contained 218.9 mgm. of nitrogen as nitrite per 100 cc. of solution.

The sporadic growth in bouillon in the various generations is evident from a glance at the chart. All the daughter cultures were not made on the same date but at different times; however the same medium was used throughout. It was thought that this periodic growth in bouillon could be explained by a loss of virulence of the nitrite-forming organism, since some of the cultures in the chart failed to produce oxidation of the ammonium salt. Perhaps the organism would produce growth in bouillon when possessing its usual activity, but when a weakness in its oxidizing power began to express itself the bouillion test would give negative results. This possibility has not been sustained by the results with a majority of the cultures. Some cultures showing unusual activity produced no growth in bouillon. However, those cultures showing low oxidizing ability in general give negative results when inoculated into bouillon. Bouillon of varying degrees of acidity and alkalinity was used and the results indicated that the neutral bouillon was equally as satisfactory as bouillon slightly acid or slightly alkaline. *Many transfers have been made from the growth in the bouillon tubes to fresh ammonium sulfate medium but in no case has oxidation resulted.* The growth secured in the bouillon was quite characteristic in all cases—first very scanty and requiring an incubation of 6 to 10 days, then giving the medium only a very slight cloudiness. It was very difficult to secure a characteristic stained preparation from the bouillon, but in general the organism was very small, less than 1 μ in length, a distinct bacillus, often appearing as a mere dot.

No explanation can be given for the fact that a few of the cultures shown in the chart failed to oxidize the ammonium salt. This failure to oxidize was not met with in the cultivation of the crude cultures but was noticeable in all the pure cultures. This indicates that the true nitrite-forming organism is aided by the contaminating forms present in the enrichment cultures.

From the results obtained in the foregoing work it is obvious that bouillon cannot be used as a test for purity of the nitrite-forming organism unless other confirmatory tests be made, such as cultivation on agar or silicic acid gel.

Winogradsky found the nitrite-forming organism in his cultures in two stages, one of which he terms "schwärmer" and the other the "free cell stage." It is doubtful if such forms were present in this work, though such might have been the case. The free-cell type was common in all the cultures, and many strains showed great masses of the organisms which resembled very closely the "schwärmer" stage; however, this latter character of growth differed but slightly from the forms found as free cells and was not considered to be a mor-
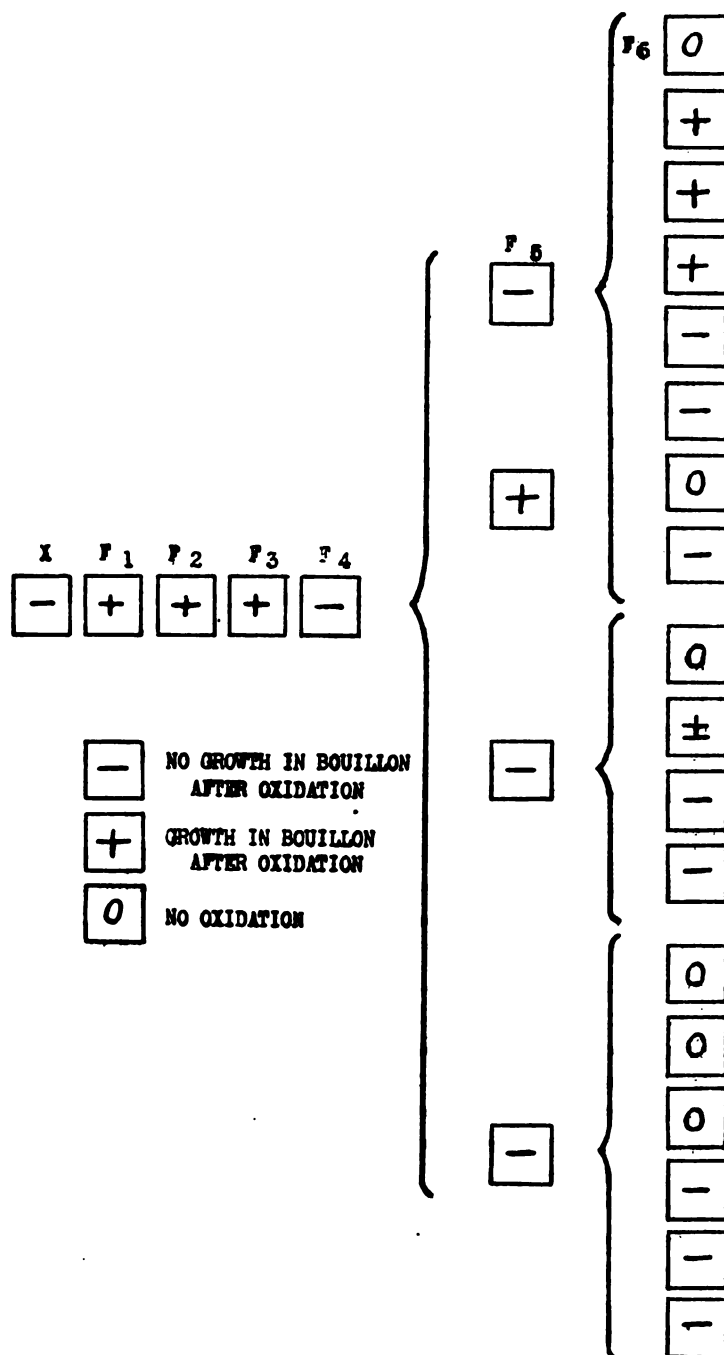
FIG. 1. CHART SHOWING RESULTS OF BOUILLON TESTS FROM NITROSOMONAS CULTURES

phologically different form, although the organisms within the group were occasionally slightly smaller.

When washed agar plates were prepared and inoculated from bouillon sterile cultures active growth and oxidation resulted in many cases. Colonies developed in very great numbers but very few of these would produce oxidation when transferred to the ammonium sulfate nutrient solution, and none would produce growth when transferred to bouillon. This inability of the organism to oxidize the ammonium salt in solution when transferred from the plates was a very puzzling problem. It was thought possible that there was a symbiotic relationship between the type growing in bouillon, if a contaminating form, and the true nitrite-forming organism. If this was the case each would lend its influence on the agar plate and when one of the forms was transferred to liquid medium oxidation would not result because of the absence of the other form. In order to check this possibility many colonies were selected from agar and silicic acid gel and transferred to flasks of the nutrient solution. These flasks were then inoculated from the characteristic bouillon growth as well as with colonies selected from Heyden agar plates which had been inoculated from a crude culture. The results in general were negative, indicating that if such symbiotic relationship existed it was destroyed by the development of the organisms on agar. It is not believed that any organism other than *Nitrosomonas* was present on the plates. The fact that *Nitrosomonas* colonies develop on washed agar and silicic acid gel, and maintain their oxidizing power on such plates, and then produce oxidation in such a small percentage of cases when transferred from the plates to ammonium sulfate nutrient solution, is explained in that the conditions met with on the plates are not entirely favorable and have brought about a weakening of the organism which is first detected when transfers are made to the original liquid medium. This necessitates the use of a large inoculum, which explains the failure in many cases to secure oxidation from a single colony.

It was thought for a long time that this growth in bouillon was the nitrite-forming organism but later results proved that such was not the case. Pure cultures have been obtained from colonies and held on hand for many generations without producing growth in bouillon. The sporadic appearance of the growth in bouillon in the early work is held to be due to contamination. This small bacillus is so closely associated with the nitrite-forming organism and is present in such small numbers that it is not consistent in showing its presence. The nutrient requirements of the organism are so slight that it is able to develop slightly in the nitrifying solution with the nitrite-forming organism.

Accumulation of nitrite in liquid cultures. The extremely high concentration of nitrite which could be withstood by the organism was noticeable throughout the work. The nitrite content of one series of cultures has already been given, one of which contained 208.1 mgm. of nitrogen as nitrite per 100 cc. of solution. Another experiment was planned to determine the concentration of nitrite which would be formed before the activity of the organism was entirely pro-

hibited. Two 1-liter Erlenmeyer flasks, each containing 100 cc. of the ammonium sulfate nutrient medium, were inoculated from an active culture and incubated at 28°C. As soon as the cultures showed the absence of ammonia by chemical test 1 cc. of sterile 10 per cent ammonium sulfate solution was added to each, and this continued until no further oxidation of the ammonium salt could be secured. Culture I always gave negative results when inoculated into bouillon, while its duplicate II always produced growth. Sub-cultures from each gave prompt oxidation of the ammonia and subsequent tests with bouillon gave the usual periodic growth outlined in previous discussion. The flasks contained magnesium carbonate in excess, so no visible growth could be seen in either. After a long period of incubation the magnesium carbonate became exhausted from the formation of nitrous acid and the solutions became clear. It was then noted that a flaky material was forming in culture I while culture II was almost entirely free from it. Sterile magnesium carbonate was then added to each culture and the additions of ammonium sulfate continued. The flaky material continued to develop in culture I and soon appeared in culture II. Stain preparations from this material showed it to consist of large masses of the *Nitrosomonas* cells, disintegrated material, and a few crystals of some insoluble salt. After no further oxidation could be secured stains of this material showed it to contain relatively few typical *Nitrosomonas* cells, but large numbers of disintegrating cells. Evidently the high concentration of nitrite was bringing about the destruction of the organism. The cultures were then analyzed for nitrite content and culture I found to contain 218.9 mgm. of nitrogen as nitrite and culture II 201 mgm. of nitrogen as nitrite per 100 cc. of solution.

Thermal death-point. The thermal death-point was determined by use of actively oxidizing pure cultures in liquid media. Tubes containing 10 cc. of 0.7 per cent sodium chloride solution were placed in the water baths at various temperatures and held until of uniform temperature throughout. Then 0.5 cc. of the culture was pipetted into each tube, the contents thoroughly shaken, and held at the temperature for 10 minutes; 1 cc. was then drawn from the tube and used as inoculum in the usual ammonium sulfate nutrient solution. All tests were run in duplicate at different times, different cultures being used. The thermal death-point was found to be between 53 and 55°C.

Longevity. The question of preserving cultures in the laboratory for relatively short periods of time is one which causes no anxiety. Active cultures in solution were capped with tinfoil and set aside in lockers in the laboratory, and after the elapse of two months the organisms were able to resume activity immediately on introduction into fresh medium or on the addition of more ammonium sulfate solution.

Test tubes containing soil were sterilized in the autoclave and after being allowed to aerate several days were moistened with very dilute ammonium sulfate solution and inoculated with the organisms. Some of the tubes were sealed with the soil in moist condition while others were left with cotton plugs.

The organisms were found to be present after 10 months' incubation at room temperature, both in the sealed tubes and those in which the soil had become air-dry, and able to bring about the oxidation of the ammonia when brought into the nutrient solution.

Field soil which had been air-dried and passed through a 100-mesh sieve and stored in tightly stoppered bottles was found to contain the organisms after the lapse of nearly 7 years.

Sensitivity to light. Cultures of both *Nitrosomonas* and *Nitrobacter* are very sensitive to light. When incubated in the laboratory neither organism will produce oxidation unless protected from the light. Direct sunlight causes complete destruction of the organism, while diffused light merely prevents their activity unless exposed for a long period of time, which proves fatal.

Contaminating forms. There are three forms which remain present in the enrichment cultures of both *Nitrosomonas* and *Nitrobacter*. On washed agar one type forms a very small light colored colony, visible to the naked eye, which is also nearly colorless when examined under the microscope. The colony has a characteristic thin and dull appearance. The other type appears to the naked eye as a very small compact yellowish brown dot, and is dark yellow or brown when examined under the microscope. Both types produce growth in bouillon. Both develop sparingly on slopes of bouillon agar, Heyden agar, and mannite agar, the latter form producing a yellow pigment on the Heyden agar. The pigment-forming organism brings about a reduction in nitrate broth with the formation of nitrite and ammonia. The other organism has no reducing action. No growth was secured when the following media were inoculated with each organism—milk, gelatin, Giltay's solution, sucrose broth, lactose broth, and urea broth.

Both organisms are stained fairly well with carbol fuchsin. The yellow-pigment former is a very small bacillus ablut 0.4 μ wide and 1 μ long, always single and never found in chains. The other is a very small coccus, or sometimes slightly oval, about 1 μ in length, often found in pairs. Both organisms are gram-negative. These two forms were eliminated before the unusual results with bouillon above recorded were secured. They both produce much heavier growth in bouillon than the type of growth observed in the bouillon-sterile series of cultures, and only a small amount of inoculum is necessary to produce the growth in bouillon.

The third contaminating form is the one found in comparatively pure cultures and the one which has given so much trouble throughout this work. It produces colonies on washed agar and silicic acid gel very similar in gross appearance to the nitrifying organisms. It produces very slight turbidity in bouillon after an incubation of several days. It is a minute bacillus easily stained with carbol fuchsin. It is hoped at a later time to give a more complete report on the morphological and physiological characteristics of this organism.

SUMMARY

The enrichment process with cultures of both *Nitrosomonas* and *Nitrobacter* was continued for a long period of time. The results with both series of cultures plainly indicated that the enrichment process could be continued for an indefinite period of time without the slightest loss of activity of the organisms. Experiments with these cultures were made to determine the number of nitrifying bacteria which they contained, the number of contaminating organisms, and the effect of various treatments on the oxidizing power of the organisms. The number of nitrifying bacteria increased from relatively few to more than 10 million per cubic centimeter of the liquid medium. Further tests by dilution and plate methods showed that foreign organisms were present in the cultures in greater numbers than the nitrifying organisms, consequently a pure culture could not be obtained by the enrichment process alone. It was found that silicic acid had no effect on the activity of the organisms. Soil extract was also found not toxic. When the extract was used to prepare the nutrient solutions the activity of the organisms was equally as great as when conductivity water was used. *Nitrosomonas* cultures withstood a concentration of 1.00 per cent sodium chloride but oxidation was greatly retarded at that concentration.

Plates and slopes of washed agar and silicic acid gel were used with success throughout the work. The gypsum block did not give satisfactory results; so its use was discontinued early in the work. The colonies which developed on either washed agar or silicic acid gel were very small, which condition necessitated the use of the microscope in all colony study. Single colonies were removed from the plates by means of a modification of the Barber apparatus. These colonies were transferred to liquid media and their activity measured by the rate of oxidation of the nitrogen salt. All colonies of *Nitrobacter* thus transferred to liquid media did not produce oxidation of the nitrite; this did not signify that the particular colony was not *Nitrobacter*, but is explained in that the organism is somewhat weakened by the growth on the solid medium, and a sufficient quantity of inoculum is not secured from the single colony. *Nitrobacter* cultures were tested for purity by inoculating into bouillon, with 0.5 cc. as inoculum. Pure cultures would produce no visible growth in this medium.

Colonies of *Nitrosomonas* transferred from plates to liquid medium usually failed to produce oxidation. This was first thought to be due to contaminating forms but this conclusion was later proven to be erroneous. When plates were inoculated from pure cultures of *Nitrosomonas* only 10 to 20 per cent of the colonies which developed would produce oxidation when transferred to liquid medium. This was due to the fact that the organism was weakened by its development on the solid medium and sufficient inoculum was not used when transferring back to the liquid medium. Those producing oxidation when introduced into the liquid medium represented those colonies in

which the organisms were not materially weakened by the conditions in the solid medium.

The bouillon test with *Nitrosomonas* was at first very confusing. The results outlined in previous pages indicate that this organism will not produce growth in bouillon. When growth was secured in bouillon inoculated from cultures of *Nitrosomonas* they were considered impure. In a careful review of the literature on the isolation of *Nitrosomonas* it is found that many investigators observed this bouillon form in their pure cultures, and some conclude that it is the true *Nitrosomonas*, which is pleomorphic. Many investigators report the presence of this bouillon form in their cultures and their work has been given more or less skeptical criticism because of such growth. The work of Stutzer and Hartleb, in regard to the organism *Hyphomicrobium*, introduces another factor which, to the present time, has not been completely worked out.

CONCLUSIONS

Pure cultures of *Nitrosomonas* and *Nitrobacter* were isolated from soil and cultivated on artificial media.

Both *Nitrosomonas* and *Nitrobacter* develop readily on plates of washed agar or silicic acid gel. The latter medium is more satisfactory in the work of isolation, disregarding its difficulty of preparation, but after pure cultures are secured washed agar can be used with success.

The colonies which developed on the plates were extremely small and required the use of the microscope in the study of their characteristics. Isolated colonies were removed from the medium by means of a modification of the Barber apparatus.

Pure cultures of either *Nitrosomonas* or *Nitrobacter* will produce no visible growth when inoculated into bouillon. In using bouillon as a purity test 0.5 cc. of the culture must be used as inoculum to give reliable results.

Pure cultures of these organisms can be maintained in liquid medium for an indefinite period of time.

The enrichment process with both *Nitrosomonas* and *Nitrobacter* can be continued for an indefinite period of time without the slightest loss of activity of the organisms. The F_{52} enrichment showed as great activity as any of the preceding generations.

Neither the enrichment process nor the securing of "super-enrichment" cultures will yield a pure culture of *Nitrosomonas* or *Nitrobacter* without the use of suitable solid media.

By careful manipulation the number of nitrifying organisms in the enrichment cultures can be increased from relatively few to a number greater than 10 million per cubic centimeter of the culture solution.

Soil extract used to prepare the nutrient solutions for the cultivation of both *Nitrosomonas* and *Nitrobacter* did not prove toxic in either case.

Sodium chloride in a concentration of 1.00 per cent was very toxic toward *Nitrosomonas*.

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PLATE 3

NITROBACTER FROM CULTURE IN LIQUID MEDIUM; STAINED WITH CARBOL FUCHSIN; × 2500

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PLATE 3

NITROBACTER FROM CULTURE IN LIQUID MEDIUM; STAINED WITH CARBOL FUCHSIN; × 2500

476
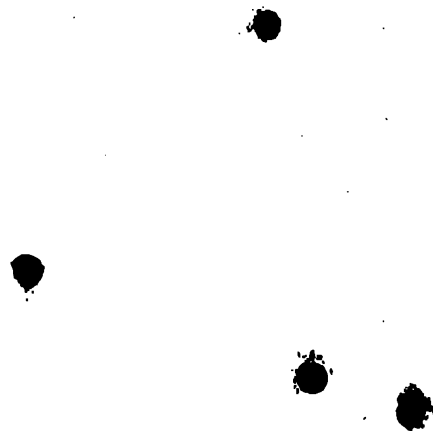
PLATE 4

SURFACE COLONIES OF NITROSOMONAS ON SILICIC ACID GEL; STAINED WITH CARBOL FUCHSIN
WITHOUT REMOVING FROM THE GEL; × 200

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PLATE 5

SURFACE COLONY OF NITROSOMONAS ON SILICIC ACID GEL; STAINED WITH CARBOL FUCHSIN
WITHOUT REMOVING FROM THE GEL; × 1200
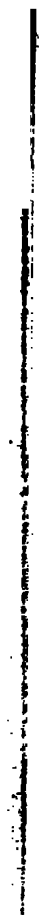
THE EFFECT OF CERTAIN NITROGENOUS AND PHOSPHATIC FERTILIZERS ON THE YIELD OF CRANBERRIES

CHARLES S. BECKWITH

New Jersey Agricultural Experiment Station

The study of fertilizers for cranberries is being carried on by the experiment stations of Massachusetts, Wisconsin and New Jersey.

The Massachusetts Agricultural Experiment Station has been conducting fertilizer experiments for ten years. Dr. Franklin (3) of that station finds under Cape Cod conditions, that although plots treated with commercial fertilizer give a slightly larger crop, the increases are not sufficient to pay for the fertilizer, and the labor expended in applying it.

The Wisconsin Agricultural Experiment Station (4), on the other hand, recommends the use of commercial fertilizers on Wisconsin bogs. It suggests the following applications per acre:

	pounds
Acid phosphate	240
Nitrate of soda	80
Sulfate of potash	80

The New Jersey Agricultural Experiment Station has been investigating the use of plant-food for cranberries in a general way since 1913 (1, 2, 5, 6, 7, 8, 9, 10). The preliminary work showed that there were three types of cranberry soil: savannah, mud and mud underlaid with iron ore deposits. The savannah soil was a sand with enough dark silt to give the whole a black appearance; the growers often call it "hard-bottom." Mud, on the other hand, consists of from 10 inches to 20 feet of peat usually over hardpan. Mud sometimes occurs on top of iron ore deposits and in such cases its composition differs from that on ordinary hardpan.

The plant-food work of the New Jersey station between 1913 and 1918 was limited to testing the effect of various plant-foods and the best sources of each ingredient. The results brought out the following six points:

(1) Both nitrogen and phosphoric acid either alone or combined gave good results on savannah soil.

(2) Sulfate of ammonia alone was an undesirable source of nitrogen.

(3) Applications of phosphoric acid gave good results on mud and mud underlaid with iron deposits.

(4) Nitrogen derived from nitrate of soda gave results easily shown in quickened vine growth during the week following the application while nitrogen

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from dried blood did not show its effect for from 3 to 5 weeks after application. The nitrogen in either case had some effect on the crop during the year in which it was applied.

(5) Phosphoric acid derived from acid phosphate was immediately available while phosphoric acid derived from rock phosphate was not available until the year following its application, but when it became available it was as desirable as phosphoric acid from acid phosphate.

(6) Annual applications of 40 pounds of nitrogen to the acre resulted in too much vine growth.

As stated above these investigations were planned to indicate the kind of plant-food needed on the cranberry bog and the desirable sources of such plant-food. During 1919 studies were started to determine the quantity of the beneficial plant-foods desirable for annual application; as well as to test the more promising new sources of plant-food. The studies were undertaken to determine the following:

(1) The optimum amount of nitrogen which should be applied annually to savannah soil.

(2) The derivation of needed nitrogen for savannah soil from a combination of mineral and organic sources.

(3) The optimum amount of phosphoric acid for savannah, mud and iron ore bottoms.

(4) The optimum amount of a tentative formula for mixed fertilizer to be applied to savannah soil. The tentative formula was made up by the station on a basis of the results of the last six years.

(5) The value of calcium cyanamide as a source of nitrogen for savannah bottom and the value of barium sulfate on cranberry soil.

1. THE OPTIMUM AMOUNT OF NITROGEN WHICH SHOULD BE APPLIED ANNUALLY TO SAVANNAH SOIL.

2. THE DERIVATION OF NEEDED NITROGEN FOR SAVANNAH SOIL FROM A COMBINATION OF MINERAL AND ORGANIC SOURCES

The object of this set of experiments is twofold: first, to determine the optimum amount of nitrogen which should be applied annually to savannah soil, and second, to compare the effect of nitrogen derived from mineral sources with that from organic sources.

Former studies conducted by this station have shown that 40 pounds of nitrogen is undoubtedly too much to apply to an acre of cranberries annually; also, in the experience of one of the growers, 10 pounds had been applied without beneficial results. Obviously, the optimum was somewhere between these amounts and the station determined to try 20 pounds and 30 pounds.

The search for the best source of needed nitrogen, from the experience of the station with a variety of substances, narrowed itself down to nitrate of soda and dried blood. Either of these substances becomes readily available

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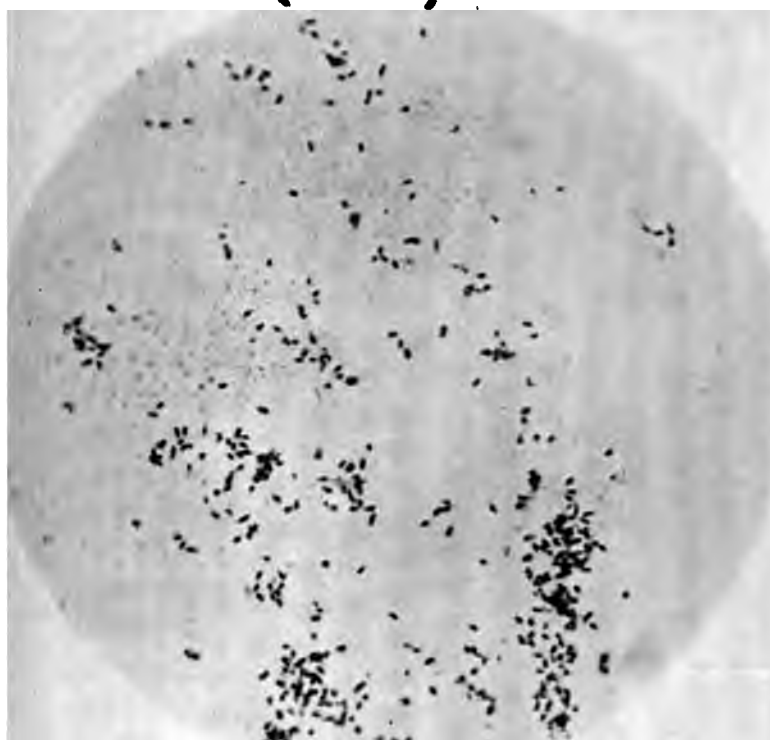
3. THE OPTIMUM AMOUNT OF PHOSPHORIC ACID FOR SAVANNAH, MUD AND FROM ORE BOTTOM

It is recognized that a sufficient amount of phosphoric acid is lacking in most cranberry soils, but the amount that may be applied economically each year is not definitely known. The study here reported was designed to throw light on this problem.

In former investigations conducted by this station phosphoric acid derived from acid phosphate gave immediate returns; on the other hand, phosphoric

TABLE 2

Results of phosphate tests; variety, Early Black

PLOT	TREATMENT	P₂O₅	YIELD					
			Savannah SB-F-P		Mud MB-F-P		Iron ore IB-F-P	
			Pounds per acre	Per cent gain	Pounds per acre	Per cent gain	Pounds per acre	Per cent gain
	lbs. per acre	*lbs.*						
1	Nothing............................		2440		7600		2340	
2	125 pounds acid phosphate..............	20	2280	−3	9660	18	2000	−10
	75 pounds phosphate rock..............	20						
3	Nothing............................		2240		8700		2080	
4	250 pounds acid phosphate..............	40						
	150 pounds phosphate rock..............	40	3720	29	9000	5	3160	90
5	Nothing............................		3520		8060		1160	
6	375 pounds acid phosphate..............	60						
	225 pounds phosphate rock..............	60	3200	−4	10140	20	1180	2
7	Nothing...........................		3120		8820		960	
8	500 pounds acid phosphate..............	80						
	300 pounds phosphate rock..............	80	3580	17	11320	16	1840	30
9	Nothing............................		3000		10900		1880	
10	150 pounds phosphate rock..............	40	3000	2	9160	16	2320	0
11	Nothing............................		2880		10820		2840	
12	150 pounds soft phosphate rock..........	40	2840	−2	8340	−9	3820	6
13	Nothing............................		2920		7640		4400	
14	250 pounds acid phosphate..............	40	3440	11	8720	18	4460	37
15	Nothing............................		3360		7340		2260	

acid from rock phosphate gave only moderate increases until after the first year, but when it began to operate in any marked degree its results were quite as good as those from acid phosphate. Rock phosphate, because of its alkaline reaction, did not leave an undesirable residue in the soil and it was the most beneficial of the materials already tested. This was used as the basis of the treatments. As rock phosphate would not become effective until the second year, a treatment was made of an equal amount of phosphoric acid derived from acid phosphate. This extra treatment was to furnish phosphoric acid

for the first year, the treatment in following years, except on plot 4, to be merely rock phosphate. The treatments and yields are given in table 2.

Series IB-F-P was located on low ground where usually there is sufficient drainage but during the current year a large amount of rainfall kept the soil saturated and the results are not the best that could be expected.

The test shows that 300 pounds of rock phosphate is not enough to cause damage. Applications of acid phosphate caused increases in yield rather consistently but rock phosphate was not active in the first year. These figures of course, are based on the one year's crop,·and the crop of the second year is expected to show more definite results.

4. THE OPTIMUM AMOUNT OF A TENTATIVE FORMULA FOR MIXED FERTILIZER TO BE APPLIED TO SAVANNAH SOIL

The station felt that it was ready to publish a tentative formula for a complete fertilizer for savannah land and determine as nearly as possible the amount needed for annual applications. It was made up on the basis of the last six years' results and is as follows:

	pounds
Sodium nitrate	75
Dried blood	75
Rock phosphate	300
Sulfate of potash	50

The first year this material was used 300 pounds of acid phosphate was added in order to have phosphate available the year of application.

The results of tests with this mixture are given in table 3.

TABLE 3

Results of tests with a mixed fertilizer on savannah soil; variety, Early Black

PLOT	TREATMENT	YIELD	INCREASE OVER CHECKS
	lbs. per acre	*lbs. per acre*	*per cent*
SB-F-C-1	Nothing	3800	
SB-F-C-2	264 pounds mixture; 176 pounds acid phosphate	4780	20
SB-F-C-3	Nothing	4000	
SB-F-C-4	528 pounds mixture, 352 pounds acid phosphate	5180	20
SB-F-C-5	Nothing	4680	
SB-F-C-6	792 pounds mixture, 528 pounds acid phosphate	6340	38
SB-F-C-7	Nothing	4500	
SB-F-C-8	1056 pounds mixture, 704 pounds acid phosphate	5200	49
SB-F-C-9	Nothing	2860	

Plot SB-F-C-9 proved abnormally poor and on this account Plot SB-F-C-8 showed a high percentage of gain, although it actually yielded a smaller crop than Plot SB-F-C-6.

That Plot SB-F-C-8 was over-fertilized was shown by the excessive vine growth on the plot and the many "runners" that appeared on top of the vines. The crop was somewhat less than on SB-F-C-6 which was in excellent condition. Plots 2 and 4 are expected to give much better yields the second year.

5. THE VALUE OF CALCIUM CYANAMIDE AS A SOURCE OF NITROGEN FOR SAVANNAH BOTTOM AND THE VALUE OF BARIUM SULFATE ON CRANBERRY SOIL

Calcium cyanamide was tested as a cranberry fertilizer in 1919. The material used tested 18.16 per cent nitrogen. The treatments were made on savannah soil on Howe berries, with the results reported in table 4.

TABLE 4

Results of tests with calcium cyanamide and barium sulfate on savannah soil; variety, Late Howe

PLOT	TREATMENT	YIELD	INCREASE OVER CHECKS
	lbs. per acre	*lbs. per acre*	*per cent*
1	Nothing..	5860	
2	120 pounds calcium cyanamide..........................	4900	−17
3	Nothing..	5900	
4	120 pounds calcium cyanamide, 250 pounds acid phosphate, 220 pounds sulfate of potash...........................	6000	2
5	Nothing..	5900	
6	120 pounds calcium cyanamide, 2000 pounds ground limestone.	4740	−20
7	Nothing..	5900	
8	120 pounds calcium cyanamide, 250 pounds acid phosphate, 220 pounds sulfate of potash, 2000 pounds ground limestone	6340	6
9	Nothing..	6040	

The results indicate that as a source of nitrogen calcium cyanamide gives unsatisfactory results in the first year. This is shown both when it is applied alone and when applied with limestone. The loss was not serious when used in a complete fertilizer but the gain over the checks is small.

6. TEST OF BARIUM PHOSPHATE AS A SOURCE OF PHOSPHORIC ACID

Barium phosphate was suggested as a source of phosphoric acid and was tested during 1919. The treatments and the results are given in table 5.

These results indicate that barium phosphate has little plant-food value for cranberries within the first year after its application.

TABLE 5

Results of tests with barium phosphate; variety, Early Black

PLOT	TREATMENT	P₂O₅	YIELD					
			Savannah		Mud		Iron ore	
			Pounds per acre	Per cent gain	Pounds per acre	Per cent gain	Pounds per acre	Per cent gain
	lbs. per acre	*lbs.*						
1	Nothing.............................		2920		7640		4400	
2	250 pounds acid phosphate..............	40	3440	11	8720	18	4460	37
3	Nothing.............................		3360		7340		2260	
4	150 pounds phosphate rock and 7 per cent barium sulfide......................	40	3560	8	7840	1	1060	40
5	Nothing.............................		3160		7920		1480	
6	150 pounds soft phosphate rock and 7 per cent barium sulfide...................	40	3240	9	7120	−2	2020	20
7	Nothing.............................		2760		6580		2000	
8	150 pounds barium phosphate...........	40	2400	−9	6440	−3	2940	−2
9	Nothing.............................		2480		6680		5880	

CONCLUSIONS

The experience of the first six years of cranberry plant-food studies has shown that general conclusions cannot be based upon the results gained with one year's crop, but the results are valuable in that they give some indication of what to expect in a general way. The following are the chief points brought out by the investigations:

1. Thirty pounds of nitrogen to the acre gave a better yield than 20 pounds per acre in the first year of the application. The vines receiving either amount were left in excellent condition.

2. Applications of a mixture of mineral and organic sources of nitrogen did not give a better crop than nitrate of soda alone during the first year.

3. The optimum amount of phosphoric acid to be applied was at least 80 pounds on savannah soil, mud bottom and iron ore bottom.

4. The optimum amount of the tentative mixed fertilizer for savannah bottom is 800 pounds, together with 500 pounds of acid phosphate in the first year. This amount of fertilizer, when applied to reasonably vigorous vines, helps to establish a strong growth and to increase the crop.

5. Calcium cyanamide is an unsatisfactory source of nitrogen, in the first year.

6. Barium phosphate is an unsatisfactory source of phosphoric acid in the first year.

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